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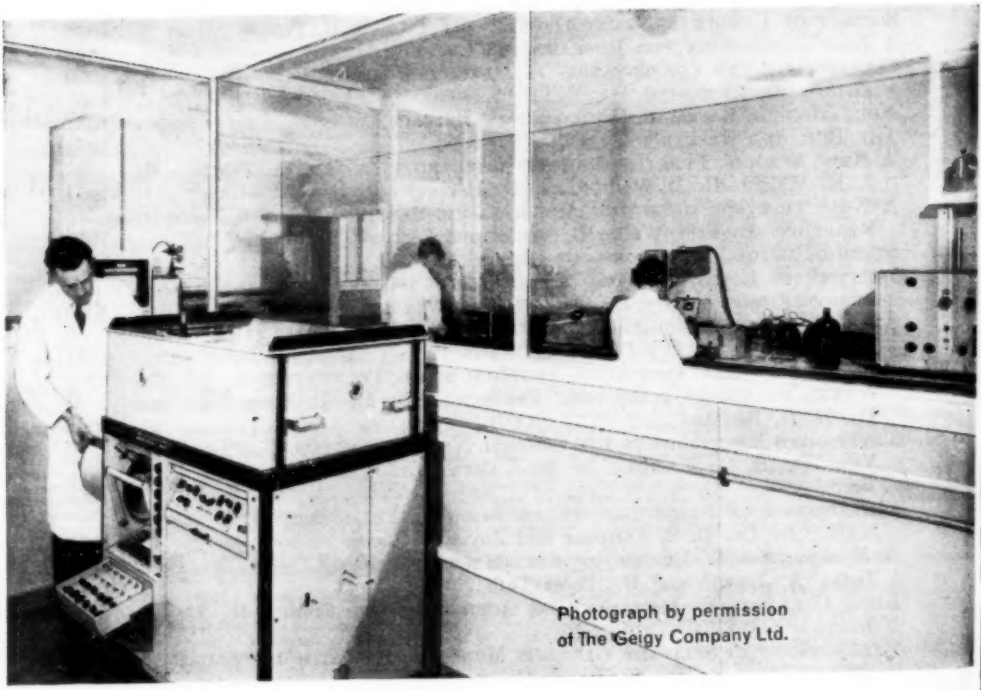

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UNIVERSITIES IN BRITAIN

THE vital importance of the task confronting the Robbins Committee on the educational system of Britain is being emphasized on all sides. The recent decision of the Minister of Education regarding the status of the colleges of advanced technology is admittedly only a temporary decision pending the report of that Committee, when more fundamental decisions will need to be taken regarding not simply the government and autonomy of such institutions but also their staffing, their size and their relation to the universities on one hand and to the technical colleges and technical schools on the other. The implications of their location do not appear to have been examined as they should have been, and there are also questions of staff and their status. Again, it is obvious that the expansion of the universities requires consideration both in regard to the balance, or rather the relations, between research and teaching and to the place of academic research, quantitatively as well as qualitatively, in Britain's organization for research. This is apparent from the recent report of the Council for Scientific and Industrial Research and also from the comment which has been made on the installation of the radio telescope at the Royal Radar Establishment rather than at a university. Nor can proposals for university expansion leave out of account particular problems, like that of mathematical education, to which much attention has been given recently. Even when such problems demand immediate remedial attention, any reorganization of higher education should be concerned to see that such crises do not recur.

For such reasons, Prof. H. C. Dent's little book, *Universities in Transition**, and the broadcast talks in the Third Programme by Sir Eric Ashby, "Universities To-day and To-morrow" (*Listener*, June 1), and of Mr. Norman Fisher, "The Future of Our Universities" (*Listener*, June 29), are timely. They are addressed to the layman, to the ordinary public, rather than to those who are engaged in academic work, and they should help to build up an intelligent public opinion on the issues involved in the expansion of the universities of Britain, and the implication of such expansion both for the national well-being and for the educational system as a whole. No less important, they help to place in a true perspective the numerous special issues arising out of such questions as apprenticeship and technical training generally, university selection and the content of curricula, even when they are presented in books of the quality of *The Complete Scientist* and Mr. W. D. Furneaux's Nuffield Foundation Study, *The Chosen Few*. Prof. Dent, moreover, provides an admirable select bibliography which offers the reader a reasonable conspectus of current discussion; as, in fact, in his limited field, does Mr. Furneaux.

Prof. Dent divides his space fairly evenly between the past, the present and the future. In the first section, he describes briefly what he regards as the most significant features in the development of universities in Britain up to 1939, stressing more particularly the rise of the modern universities. In the second, he outlines in some detail the expansion of universities between 1945 and 1960 and the wartime planning which made that expansion possible. Naturally, these two sections are largely recapitulation to anyone familiar with the subject, but even so, Prof. Dent contrives here and there to emphasize points that are often overlooked and bring out their relevance in the present situation, and he indicates weaknesses which we should be at pains to avoid in any future planning.

It is in his third section, when he examines critically some of the more important problems thrown up or made more acute by the present expansion, that Prof. Dent's own judgment finds free expression, and the order of priorities which he indicates deserves careful consideration. First, however, he suggests that the difficulties confronting British universities to-day can be divided, broadly, into two groups: those concerning internal administration and the conduct of university activities, and those posed by the ethos, intentions and organization of society. Most of the problems in the first group can, in any event to some extent, be solved by the action of the universities alone. None of those in the second group can.

This, of course, is a fundamental reason why it is so important that there should be a really informed public opinion as to what the universities are doing and what is involved in their expansion, although this is frequently unrecognized even by those who are foremost in pointing out that universities are social institutions. The fundamental problem, however, underlying and determining the character of all the others, Prof. Dent rightly maintains, is what kind of institution the university of to-morrow should be, or in other words: What is its primary purpose? He agrees with Sir Eric Ashby that the social purpose of the European university has, within broad limits, remained constant throughout the ages, namely, to be society's principal means for conserving, disseminating and advancing academic knowledge, and, consequently, to be its principal source of supply of persons highly trained in academic disciplines; but he believes that in Britain the nature and scope of that purpose need reconsideration and some of its elements realignment.

Prof. Dent points out, for example, that there are now many institutions devoted solely to research, and the university is no longer the sole institution which accepts the advancement of knowledge as a corporate responsibility. Quite apart from the desirability of the colleges of technology extending their research activities, there are the independent research institutions, and it is significant that, in passing, Prof. Dent stresses the need for more co-operation and co-ordination between various institutions engaged in research. In this context, indeed,

* *Universities in Transition*. By H. C. Dent. Pp. 176. (London: Cohen and West, Ltd., 1961.) 15*s*. net.

he suggests that broad fields of learning might be allocated as a special responsibility to particular institutions.

There are obvious difficulties in such an arrangement, but it may well be worth exploring, and it is interesting to note how far Prof. Dent's brief discussion is in line in a British context with the findings of a recent report of President Kennedy's Science Advisory Committee. Moreover, he accepts the impracticability of divorcing university research from teaching, and his discussion of research is designed primarily to elucidate what it is the university can offer that is unique. Even teaching at the highest level is likely, for example, to be in the future increasingly shared between the universities and other types of institution, including the colleges of technology.

Prof. Dent suggests that the answer may be found in the idea of community; not, indeed, in terms of the medieval idea, but in terms of an environment which affords, or rather compels, a wide range of academic and social experience of a particular kind. In consequence, a university must possess coherence, and the fragmentation and departmentalism which are sometimes encountered must not be ignored but removed. This fundamental question thus leads him to consider what conditions are necessary to facilitate a sense of community and the prospects that such conditions will, in fact, be accepted and satisfied.

It is not surprising to find that Prof. Dent believes that there should be a limit to the expansion of particular universities, and that he agrees with the Association of University Teachers that there should be a deliberate policy of preserving the pattern of university life found in the smaller and medium-sized universities. In consequence, he advocates the creation of new universities and university colleges, the diversion of considerable numbers of students who would otherwise enter universities into colleges of technology or technical colleges—it is not quite clear which he means—and the shedding by universities of some subjects now in their programmes. He questions outright whether purely professional subjects should any longer figure in university programmes, and suggests that at least all purely professional studies and activities should be transferred to other institutes, possibly associated with the universities after the fashion of the teaching hospitals or the teacher-training colleges, leaving the universities with the sole task of teaching the academic disciplines which are the bases of professional activities.

That is a proposal which Prof. Dent recognizes is likely to be unpopular; but it should at least stimulate some of the constructive thinking that is needed outside as well as inside the universities. If it should leave the universities with a task that is manageable in respect both of teaching and research, the considerable readjustments that professional institutions would be required to make might be well worth while. Furthermore, it would seem likely to meet some of the criticism that has been levelled at the education of the chemist and physiologist by Prof. T. L. Cottrell and Prof. J. A. B. Gray, and might offer the universities more room for adjustments in the content of studies to meet the needs of to-day and encourage breadth as well as depth in the education of scientists and technologists. At least it should give the universities the opportunity to impart something of the ideal of public service which is as essential a part of a professional code as technical competence.

Prof. Dent's next emphasis is on the quality of a student's private life. This, he rightly observes, can make or mar a student's university career, and he sees no adequate substitute for the community life of a college or hall of residence. He does not forget that there are sometimes disadvantages and defects which initiative and imagination could remove, but he challenges outright the belief that halls of residence are in general too expensive.

On the selection of students, Prof. Dent is much less detailed than Mr. Furneaux, but he is no less emphatic as to the need for some sort of clearing-house, as has now been recommended by the *ad hoc* Committee on Procedure for Admission of Students of the Committee of Vice-Chancellors and Principals, and the need for better use of school-leaving examinations and revision of procedure. He emphasizes, moreover, the importance of proper provision for the needs of overseas students, and thinks that attention to some of the factors which affect, often powerfully, the quality of a student's life might reduce the wastage and proportion of failures among those who secure admission.

This major criticism is, however, rather of the Government than of the universities. The universities cannot hope to offer their students all that a university education ought to imply without adequate financial resources. To do so, Prof. Dent observes, means something like doubling the present annual grants for recurrent expenditure, with *pro rata* increases as the number of students increases; and, no less essential, a sufficient increase in non-recurrent grants to provide adequate residential accommodation, equipment and buildings, including library as well as laboratory facilities and lecture rooms and provision for social and recreative activities. In this connexion it may be noted that Prof. P. M. S. Blackett has since expressed the concern held by many as to whether in submitting their applications to the University Grants Committee for 1962–67 the universities have made anything like sufficient provision for the cost of the scientific research which is their responsibility, the total cost of which could be about £14 million a year.

Two problems specifically mentioned by Prof. Dent in this connexion are those of specialization and breadth of education, which, as already noted, concentration on academic rather than professional disciplines might do something to ease, and the distractions from academic studies caused by sundry matters, including vacation employment. He suggests indeed that an inquiry into the entire range of university learning and teaching, including the content of syllabuses and the length of undergraduate

courses, is overdue; and he points to the desirability of giving those appointed to a university academic staff who have no previous experience of teaching, particularly if young, some instruction in the arts of lecturing and handling seminar and tutorial groups, etc., in the psychology of learning and of later adolescence, the general structure of the English educational system and the rudiments of knowledge of one or two other national systems.

This is essentially an administrative matter. A second point, the relative place of research and teaching in a university teacher's life, is not, though in the first place Prof. Dent suggests that the relative load of teaching and research and the staff/student ratio should be the subject of authoritative inquiry. It is urgently necessary to-day to use the services of every university teacher to the very best advantage, and this is not possible so long as the claims of research and teaching are regarded as conflicting. Prof. Dent believes, indeed, that every member of the academic staff of a university should be constantly engaged in research, but not necessarily, nor perhaps even usually, with the idea of immediate publication; though he equally believes that the primary duty of most university teachers to-day is to teach as well as possible, and for this they must continually enrich their knowledge and understanding of their subject.

The wise distribution of a university's resources depends, of course, on the quality of its administration, but though Prof. Dent, like Sir Eric Ashby, is critical of some procedures, he does not think that any major alteration in the structure of the existing types of government would be timely. He urges some delegation of responsibility from the vice-chancellor, some relief of professors from administrative responsibilities and greater participation in academic government by members of the non-professorial staff; he also insists that it is essential to distinguish clearly between political independence and academic freedom, between the university as an institution in and of society and the methods by which that institution performs its particular and unique task. Political independence of the State the British universities no longer possess, if they ever had, but Prof. Dent does not believe that academic freedom should be limited to full responsibility for its own appointments, the organization of its courses, and the character and standards of the instruction given within a university. He is convinced that the university should also have absolute control over the admission, examination and expulsion of students; the final voice in the distribution of public funds received for recurrent expenditure; and the absolute right, subject to freely negotiated agreements, to determine what forms of research and what research projects it will undertake or approve.

All these rights Prof. Dent holds to be necessary if the university is to undertake freely, imaginatively, and flexibly its unique task which, with Sir Charles Grant Robertson, he sees as the starting point of a new renaissance, for which it creates spiritual and moral power. That belief is at the heart of his plea for ensuring that the university remains a community the members of which wrestle with the fundamental problems of life as well as pursue their intellectual tasks together, and, by and large, it seems to be accepted by Mr. Norman Fisher in his broadcast talk, when he expresses the hope that British universities of the future will live up to what was said of medieval universities by Hastings Rashdall: "Their greatest service to mankind was simply this, that they placed the administration of human affairs . . . in the hands of educated men".

Apart from this, in their broadcast talks, Sir Eric Ashby and Mr. Norman Fisher have little to say about the purpose of a university. Sir Eric is concerned chiefly with the problems which staff and students present in the proposed expansion to 170,000 places: the matter of buildings he dismisses as not an educational problem, rather more perfunctorily than Mr. Fisher approves; though it is plain enough that Sir Eric requires the existence and provision of satisfactory buildings, including halls of residence, libraries, etc., just as much as Prof. Dent or Mr. Fisher. The provision of staff of high quality he recognizes as the key problem: in his view the supply of students of high enough quality offers no difficulty, nor does he suggest that plans for university expansion in Britain will or should offer every young person capable of obtaining a first degree at a university the chance to do so. On the contrary, he points to the alternative avenues of higher education, and suggests that pressure on university places might well be relieved if teacher-training colleges and the colleges of technology were allowed to award a degree instead of a diploma.

This appears to be in line with Prof. Dent's views, as also are Sir Eric Ashby's views on research and teaching, a matter which he points out bears vitally on the problem of attracting staff of high quality. Some changes in the criteria for academic success are suggested for that reason alone. Size and location are dismissed as smaller matters, provided a university has space to be a coherent community with staff and students living, preferably, within walking distance; this again is not seriously contested by Mr. Fisher, beyond the comment that there are weighty arguments for limited size, or in any event, in big universities, a system of devolution. The question of academic government is regarded by Sir Eric as of critical importance, though he does not admit that it is necessarily a matter of size. He challenges, indeed, the existing pattern of academic organization of knowledge and suggests that some of the new universities should eschew altogether the words 'faculty' and 'department', and invent some new system even if it is too much to expect abolition of internal frontiers in the older universities. Like Prof. Dent, however, he insists that a university is a society, not a hierarchy, and that it is essential to associate the main body of university teachers with the making of academic decisions, and not leave it in the hands of some 1,200 professors. Nor does he believe that the revision of university problems is a difficult problem to solve.

XUM

Mr. Fisher has rather more to say about the purpose of a university, emphasizing the importance of a university giving its students something much more than professional skill; his suggestion for the development of graduate schools is intended to assist the university to perform its function of the transmission of knowledge as well as the extension of its frontiers, and to bridge the gaps between one specialized field of knowledge and another. He, too, sees the need for radical changes in the organization of existing universities, in recruitment of staff and in the system of promotion. By and large, Sir Eric Ashby and Mr. Fisher in their broadcast talks and Prof. Dent in his short volume provide the Robbins Committee with plenty of critical comment on important matters.

The chief task of the Robbins Committee can be described shortly as to recommend what new educational institutions are needed, what they should do and where they should be situated. The sound academic arguments for not pushing individual universities above the size of 4,000–5,000 students are apparent in these papers, and the new universities already specifically contemplated will provide some 15,000 places. A similar number may well be available in the colleges of technology, particularly if such colleges are enabled to offer a degree instead of a diploma in technology, and this might well be preferable to increasing the places in university departments of technology. Similarly, the suggestion regarding the teacher-training colleges, taken in conjunction with Prof. Dent's suggestion as to professional studies, might help. Meanwhile, however, the universities themselves should look at such matters as their degree structure in relation to catering for more general studies, the adjustment of the balance between research and teaching to the advantage of teaching, and the ways and means of meeting the residential and tutorial needs of the increasing numbers of students drawn from homes where there is no tradition of culture or reading. Whatever recommendations the Robbins Committee may make —and they should cover many institutions like technical colleges, professional institutes, and the schools themselves, as well as colleges of technology and the universities—so far as they affect the universities, it will be for the universities to decide on the methods of implementation, even if it is for the Government to will the means. The shape of at least some of the recommendations can already be forecast with confidence, and the universities should push ahead with their plans and proposals for reform and development.

HOPES OF TO-MORROW

IT is disquieting that, despite all their technical skills, scientists so often have become the dupes of opportunist politicians. How adroitly Adolf Hitler manipulated them to his service. He is not the last of his species. In both halves of our polarized world scientists and technologists are to be found harnessed to the band-wagon of some capricious potentate who has succeeded in capturing the machinery of government. The Prime Minister, in his address to the Royal Society of London at the Tercentenary Banquet, claimed that politicians were the only amateurs left. Perhaps it is not too mischievous to suggest that this amateurish pursuit has incredible professional consequences. In general, scientists tend to leave their fate much to the whims of politicians. They are unconcerned with the world outside their research establishments; indeed, it is often a logical consequence of their training. To claim, as some do, that such unawareness is a virtue in itself is rather absurd.

Recently the Minister for Science raised the spectre of a languishing land, if Britain did not resort to automation more avidly*. Countries with a lower standard of living are mastering technical skills more and more. Lord Hailsham stated that, without automation, Britain will not command competitive power, but be priced out of the market. To avert this, the country must seize the lead in technology of production and be determined to remain one jump ahead of emergent countries in technical skill. He asserted that vast changes in the whole system of education would have to be made. For example, he pointed out that there are "vital questions in the field of human sciences" which are but weakly dealt with at universities and there is a dearth of management science.

It cannot be denied that new ideas in technology are necessary to prevent a mature economy from stagnating. That automation is essential for improving technology is certainly valid too. However, to suggest that national prosperity hinges on stressing automation and that education, particularly at schools and universities, be perverted to satisfy apparent technological demands of the moment is a sorry exaggeration. What may seem of paramount urgency to-day will to-morrow perhaps not seem so important.

Economic patterns are complex. A feature of a highly industrial, capitalistic economy is its sensitivity to trade-cycle fluctuations. This tendency appears to be related to the 'roundaboutness' of production: the greater the number of sequential steps between raw material and finished product, the keener is the sensitivity. Ultimately, the most powerful determinant of economic climate is what industrial and commercial management feel about the future. It is related to the temperament and emotional patterns of individuals.

To design education, particularly university education, so that scientists will acquire the skill to conjure

* Conference on Automation and Computation, Harrogate, June 27, 1961.

up automatic devices to meet the expediency of the moment could, if overdone, have dire results. For example, it is possible to place too much emphasis on the type of mathematics used for obtaining solutions by electronic computation. Numerical methods on a computer usually will provide an answer, when a function is not easily integrable by analysis or say, a troublesome differential equation has to be solved. With inadequate training in analytical mathematics, the scientist will shirk an attempt at explicit solution; he will resort to a solution with a computer. The result is found speedily, but lacks the sophistication of an explicit form. The functional form of solution might have suggested startling insight into the physical significance bearing on the problem. Use of a computer tends to block such discovery.

Lord Hailsham's exhortations concerning problems of living, the vital questions, need to be examined. It might be well if these could be treated by using 'human science', as he suggests. But unhappily (or happily) human emotions do not lend themselves readily to rational formation. They are frankly, but fascinatingly, inconsistent. "Nature is not bound by reasonableness", as D. H. Wilkinson (professor of experimental physics at Oxford) has said. It is interesting to recall Lord Hailsham's presidential address to the Classical Association in Birmingham on April 13. There he stated that a solution to our modern perplexities, our aberrations in politics and morals, in æsthetics and philosophy probably lay in establishing again "the real validity of our moral and æsthetic value judgments symbolized in the classical culture in which we were steeped in our youth". This seems a better approach than 'human science'.

As we plan to change curricula to satisfy the technological demands of our age, let us consider likely hazards to the nation. Without knowledge of non-technological aspects of living, the adults of to-morrow will be vulnerable. They will be manipulated and beguiled. It was Stendhal who, in an article to the *Paris Monthly Review**, June 1822, wrote rather provocatively, that a really useful 'science' a man can learn is the science of knowing the motives of men's actions. When you know these, you can try to cultivate in people other motives which will lead them to act in such a way as to bring about your own happiness. It does seem that men often lie when they speak of their motives. One must try to penetrate and undermine such untruths.

The art of knowing life is acquired by involvement with the full range of human passions. One must live. Always love and hatred will move men. Personal experience sometimes teaches. There are other sources. Our literature is rich in expression, "from the whispering of affection to the raging of madness" (Nietzsche). Not from the classics alone but from the genius of those with heightened sensibility in any age is the noble art of understanding human nature to be gleaned. It is likely to be withheld from those who persistently proffer their punched programmes to our latter-day Oracle of Delphi, the computer. We need men of science who command *savoir-vivre* and who have a degree of empathy. How encouraging it is to read in *The Times* of June 28 that in the French baccalauréat examination, the entrée to universities there, respect is still shown for the irrational element. Even those candidates who plan to read science at the university might in their examinations be required to produce a dissertation on subjects such as: "What is the nature of æsthetic pleasure ?" or "Are we responsible for our passions?"

So let us not be carried away by excessive enthusiasm for the expediency of to-day, but be prepared for the demands of to-morrow. Then even while:

> "Things fall apart ; the centre cannot hold,
> Mere anarchy is loosed upon the World.
> The Blood-dimmed tide is loosed, and everywhere
> The ceremony of innocence is drowned," (W. B. Yeats)

we may have an abiding confidence to judge and judge wisely. For "a fool sees not the same tree as a wise man sees" (W. Blake).

* *Selected Journalism from the English Reviews by Stendhal, with Translations of other Critical Writings*, edit. by Strickland, G. (John Calder, London, 1959).

RESPIRATORY METABOLISM IN PLANTS

Respiratory Metabolism in Plants
By Prof. Harry Beevers. (Row-Peterson Biological Monographs.) Pp. xi+232. Evanston, Ill.: Row, Peterson and Company, 1961.) 3.85 dollars.

THE title chosen for this book is meant to indicate that it does not attempt to cover the whole study of plant respiration, but is concerned only with the biochemistry of its reaction sequences. This is a rapidly developing field subject to annual inspection in various reviewing journals, but this is by no means to say that the present volume is supererogatory. It is not easy for the young student to pick the (more or less) permanent wheat from the ephemeral chaff in annual reviews. A considered statement of work from the past couple of decades is a sounder foundation, and nobody is better qualified to provide such a foundation at the present time than Prof. Beevers, who has himself made notable contributions to this work.

He has aimed at and succeeded in providing a factual statement of present knowledge with a minimum of controversy and 'interpretation' of dubious data. He covers the sequences and cycles of reactions during the production of pyruvate from glucose; the utilization of pyruvate in its various aspects, and oxidation systems and their constituent electron-transfers. These are treated at some length, and then there are briefer accounts of the energetics of respiration and the intracellular localization of respiratory enzymes.

The seventh chapter, on the regulation of respiratory-rates, seems to step a little outside the author's declared intention and to involve him rather more deeply in controversy. The remaining chapters deal with the relation of respiration to other physiological processes such as salt uptake, nitrogen metabolism, etc., and make interest ng reading.

The book has been well produced in paper-back form at a price which should not put it out of the reach of its most important customers and beneficiaries—biology students. W. O. JAMES

CIVIL ENGINEERING

Civil Engineering Reference Book

Edited by J. Comrie. Second edition. Vol. 1 : Pp. xiii + 518. Vol. 2 : Pp. xiii + 527. Vol. 3 : Pp. xiii + 599. Vol. 4 : Pp. xiii + 392 + lxii. (London : Butterworth and Co. (Publishers), Ltd., 1961.) £17 17s. 0d. the four volumes.

A REFERENCE book dealing with so vast a subject as the science and practice of civil engineering would assume enormous proportions if the objective was to provide a self-contained treatment of every aspect of the subject for the engineer. Its proportions would, however, be more modest if it contained a brief review of each aspect of the subject with adequate references to specialist publications. This work in four volumes, by some fifty-six contributors, appears to fall somewhere between these possible forms, though its aim (as stated in the introduction to the first edition) is to give the civil engineer a concise presentation of the fundamentals of the theory and practice of all branches of the subject.

There is a tendency, in those chapters involving mathematical analysis, to deal with matters of detail rather too briefly for the practising engineer. This is remedied in some instances by numerous references to specialist works as, for example, in the chapter on hydraulic power plants. In other instances, however, as in the chapter on strength of materials, the references given are not always adequate for the sophisticated mode of treatment adopted. (The confusion of the mathematical expressions for the strain energy and complementary energy of linear systems in this chapter is noteworthy.)

Chapters on mathematics, statistics, physics and chemistry, mechanics, mechanics of fluids, strength of materials, thin slabs and shells, surveying, building materials, cements, measuring appliances and general tables are contained in Volume 1. Volume 2 deals with soil mechanics, site investigation, foundations, hydraulic power plants (for example, dams, conduits and turbines), overhead transmission lines, tunnels and tunnelling practice, land drainage, hydraulics of rivers and canals, coastal engineering and explosives. Structural theory and practice relating to metal (including welding and welded construction), concrete, timber and brick and masonry construction, including buildings and bridges, is the subject of Volume 3. The fourth volume is concerned with waterways, highways, railways, airports, harbours and docks, water supplies, sewerage, civil engineering in town planning, works construction, specifications and quantities, fire protection of buildings, legal notes and patents (British). Extensive revision of the contents of the previous edition has been carried out, and the chapters on hydraulic power plants, overhead transmission lines, structural concrete, prestressed concrete, masonry and brickwork and aesthetics of bridge design are new.

It is surprising to find that such an important topic as the plastic theory of the ultimate load-carrying capacity of ductile metal structures is confined to a few non-mathematical paragraphs in the chapter on steel building structures, in view of the detailed treatment of many other aspects. (The chapter on the theory of structures is devoted entirely to an attempt at a comprehensive treatment of the various so-called methods of analysing frameworks in the elastic condition, and is thoroughly conventional.).

With the exception of the chapters on surveying, materials and measuring appliances and the tabulated data, the value of Volume 1 seems questionable. Almost any attempt to deal with subjects such as mathematics and mechanics adequately and in directly useful form for the engineer, within the compass of a single chapter as in this volume, would meet with very limited success. Moreover, there is no difficulty in obtaining good text-books in English on basic aspects of the engineering sciences. Works on engineering design and practice are much less common, however, and it would appear that the value of this book as a whole lies more in its presentation of this kind of matter than in the abbreviated accounts of theory which it contains. Even though a book of this kind requires fairly frequent revision (at least every ten years) to take account of progress, the appearance of a second edition is some indication of its appeal to the practising engineers for whom it is written.

T. M. Charlton

THE BIOLOGY OF MICRO-ORGANISMS

An Introduction to the Biology of Micro-organisms

By Lilian E. Hawker, A. H. Linton, B. F. Folkes and M. J. Carlile. Pp. 452 + 50 plates + 48 figures. (London: Edward Arnold (Publishers), Ltd., 1960.) 35*s*. net.

THE study of micro-organisms is wide-ranging and rapidly developing. This is partly because of the increasing recognition of their economic importance as agents in the manufacture of useful products, in soil fertility, and as agents of disease and spoilage; and partly because the study of microbial physiology has much to contribute to cell physiology in general. The rapid rates of reproduction of many micro-organisms make them extremely valuable tools for the study of many phenomena of general biological importance.

There are very few books which attempt to give between one pair of covers a conspectus of the morphology, physiology and ecology of the major groups of micro-organisms. Therefore this book serves a very useful purpose as a general introduction to this wide subject, without attempting to be a complete text-book of microbiology (if such be possible). It is divided into three sections: (1) morphology and life-cycles of micro-organisms, with chapters on bacteria, fungi, protozoa and slime-moulds, algae, viruses (148 pp.); (2) physiology and metabolism (118 pp.); (3) ecology of micro-organisms, including the microbiology of soil, air and water, animal and plant pathogens, the microbiology of food and other commercial products, and industrial fermentations (140 pp.). There are an appendix (20 pp.) on some techniques used in the study of micro-organisms, and an index (26 pp.).

The book is well illustrated with many photographs and diagrams. Each chapter ends with a well-chosen list of books and articles for further reading. The book should be very useful to undergraduate students of microbiology and related fields; and to postgraduates in other sciences who wish to have in convenient form a concise survey of microbiology—of the biological forms concerned, their physiology, and the numerous fields of microbial activity. The book is well printed and well produced, and is extremely good value, by modern standards, for its modest price.

B. C. J. G. Knight

WORLD-WIDE CIRCULATION OF AIR WITHIN THE STRATOSPHERE

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AND
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Introduction

IN 1949, Brewer[1] postulated a meridional circulation in the stratosphere to account for the low humidity measured in the lower stratosphere over southern England. At that time, the humidity measurements had been made up to heights of 42,000 ft.[2,3] using *Mosquito* aircraft of the British Meteorological Research Flight fitted with the Dobson–Brewer frost-point hygrometer[2]. These results showed that the humidity mixing ratio invariably fell rapidly with increasing height just above the tropopause. There was also some evidence, in the case of low tropopause heights, that the mixing ratio levelled off at a value of about 0·002 gm./kgm. at heights well within the stratosphere. The humidity measurements were later extended to heights of 50,000 ft. using *Canberra* aircraft[4,5]. These further data confirmed the earlier work and showed that the humidity mixing ratio steadied off at a value of about 0·002 gm./kgm., corresponding to a frost-point of about − 80° C. at 50,000 ft. This gave further support to Brewer's hypothesis that air rises through the equatorial tropopause, which acts as a cold trap since the air temperature there is about − 80° C. This dry air then moves horizontally polewards and finally sinks in the middle and high latitudes.

Dobson[6] extended this circulation model to account for seasonal variations in the total ozone over temperate and polar regions. He suggested that the cold pool over the winter pole stores ozone-enriched air which arrives by the meridional circulation from ozone-producing regions. This air subsides into the lower stratosphere of temperate latitudes in the late winter or spring and so accounts for the spring peak in the total ozone observed there.

The spring maximum of fission product fall-out from nuclear weapon tests was first attributed to the 'Dobson–Brewer' circulation by Stewart *et al.*[7]. The peaks in the fall-out distribution at temperate and high latitudes were also explained by this meridional circulation feeding the radioactive debris into the lower temperate and polar stratosphere where it becomes available for transfer into the troposphere.

However, some recent observations of the humidity and the tritium concentrations at heights of about 90,000 ft. over England[8] show that the 'Dobson–Brewer' circulation does not reach these heights. Also, Feely and Spar[9] have found that the world-wide distribution of the unique tracer tungsten-185 in the stratosphere up to 70,000 ft. is best explained by lateral turbulent mixing, rather than by large-scale meridional flow.

It is the purpose of this article to discuss these recent data and to describe a modified 'Dobson–Brewer' stratospheric model which is not only consistent with these new results but is also in accordance with other data on the distribution of radioactive debris, water vapour and ozone within the stratosphere.

Humidity Data

Measurements of the humidity of the air, the tritium content of the water vapour, the carbon-14 abundance of the carbon dioxide, together with the deuterium/hydrogen ratio of the hydrogen of the water vapour and the carbon-13/carbon-12 and oxygen-18/oxygen-16 ratios of the carbon dioxide have been made in recent years[8] at altitudes of 80,000–100,000 ft. over southern England. These measurements were made by collecting samples of the carbon dioxide and water vapour of the stratospheric air by means of a cooled vapour-trap, which was carried to these heights by balloon. From the relative amounts of water vapour and carbon dioxide collected, the humidity mixing ratio of the stratospheric air was deduced.

Table 1. HUMIDITY RESULTS ON FLIGHTS OVER SOUTHERN ENGLAND

Date	Height (ft.)	Humidity mixing ratio (gm./kgm.)
2.5.58	89,000	0·044
13.5.59	91,000	0·045
5.6.59	80,000	0·009
15.6.59	93,000	0·049
18.6.59	90,000	0·077
7.7.60	89,000	0·034
11.9.60	99,000	0·035

On the flight of 18.6.59, the balloon ascended through a thin layer of cloud, which may have resulted in water being deposited directly on to the sampling apparatus. This may account for the comparatively high humidity found on that flight.

The one measurement at 80,000 ft. on 5.6.59 is the driest (0·009 gm./kgm.) and may mean that a transition from dry air below to moist air above occurs at about this height.

It is not proposed to compare these humidity data with other measurements in great detail since this has already been done for the 1958 result[10]. However, since that publication, not only have the subsequent flights confirmed the presence of moist air above 80,000 ft., but also much independent evidence in broad agreement with these results has been accumulating. Measurements[11,12] on the infra-red spectrum of the Sun using spectrometers carried to altitudes of about 90,000 ft. by balloon have been interpreted to confirm the presence of moist air above a comparatively dry lower stratosphere. Similar measurements[13], using infra-red spectrometers borne by aircraft at heights up to 65,000 ft., lead to the same interpretation. Automatic frost-point hygrometer balloon soundings[14] up to 100,000 ft. show a similar humidity profile.

These humidity data are inconsistent with the thesis that the stratosphere above 80,000 ft. over middle latitudes receives air via a 'Dobson–Brewer' type of circulation.

Tritium Concentration

The tritium concentration of the air found over England between 1956 and 1960 using the vapour-trap technique[8] is reproduced in Fig. 1. This tritium

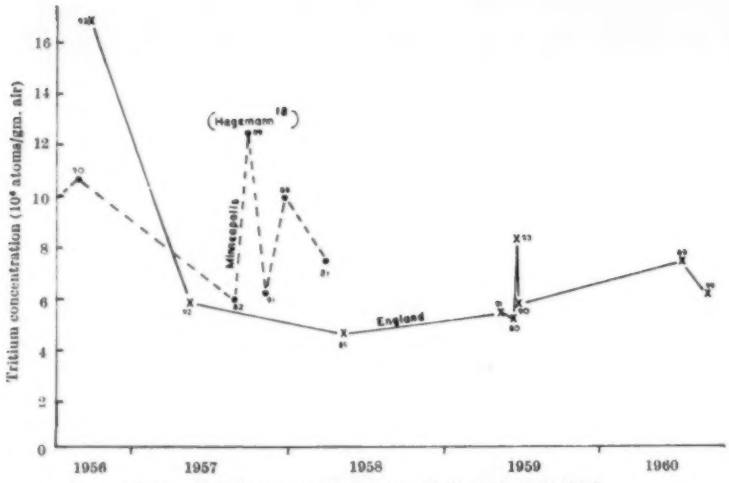

Fig. 1. Variation in the concentration of tritium above 80,000 ft.

was all contained in the hydrogen of the collected water vapour. The figures by each point give the height of the sample in thousands of feet. Also plotted are the only other comparable measurements[18] made at similar latitudes (over Minneapolis, United States) using a somewhat different technique. The bulk of this tritium has been produced during thermonuclear explosions.

Fig. 1 shows that the concentration of tritium over England since the termination of weapon testing in the autumn of 1958 has been remarkably steady and any possible large variation must have been of short duration between sampling times. The fact that the tritium concentration at 90,000 ft. has not fallen during this period suggests that the air at these heights exchanges only slowly with that at lower altitudes. This is consistent with a 'Dobson–Brewer' meridional circulation only if circulation reaching these heights is very slow. The associated humidity data, however, show that the dry circulating air does not reach 90,000 ft.

Tungsten-185

Evidence that the 'Dobson–Brewer' model is not fully compatible with the world-wide distribution in the stratosphere of late 1959 of the unique tracer tungsten-185, has recently been published by Feely and Spar[9]. The tungsten-185 was injected into the lower equatorial stratosphere at a latitude of about 12° N. during mid-1958. The maximum tungsten-185 concentration has remained at the latitude of injection near the equator above 70,000 ft., while the transfer into the lower stratosphere of higher latitudes has been quite rapid. Within one or two months of injection, tungsten-185 was found in the stratospheric debris falling out at temperate regions[15,16]. The height of maximum tungsten-185 concentration decreased progressively with increasing latitude, while the concentration compared with that at the injection latitude similarly decreased. Feely and Spar[9] suggest that lateral turbulent mixing with the possibility of some gravitational settling can best explain this tungsten-185 distribution. However, this explanation does not account fully for the observed ozone distribution, or the occurrence and maintenance of high humidities at 90,000 ft. compared with 50,000 ft.

The Requisites of a Stratospheric Model

An acceptable model of the stratosphere must be consistent with the following information pertaining to the stratosphere: (*a*) the reported world-wide distributions within the stratosphere of the following tracers: (i) tungsten-185, (ii) strontium-90, (iii) carbon-14, (iv) ozone; (*b*) the high humidities at 90,000 ft. as compared with those at 50,000 ft. in the temperate stratosphere; (*c*) the lack of a decline since 1958 in the tritium concentration in the middle stratosphere over England; (*d*) the latitudinal distribution of the radioactive fall-out of stratospheric origin reaching the ground; (*e*) the spring peak of fall-out and total ozone in temperate and polar zones; (*f*) the rapid transfer of material from the lower equatorial stratosphere to that of temperate zones, and the much greater times required for the rhodium-102 debris from rocket detonations above 100,000 ft. in the equatorial stratosphere to reach the lower extra-tropical stratosphere; (*g*) the various residence times

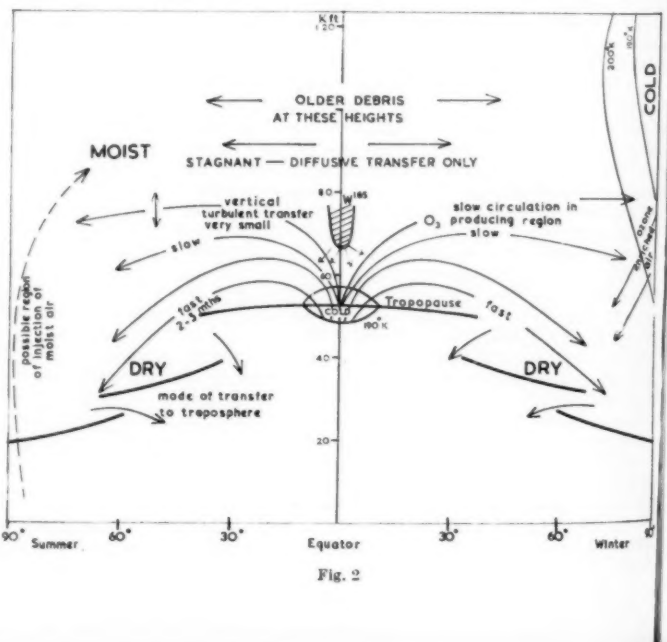

Fig. 2

of stratospheric nuclear debris depending on the latitude and height of the source.

The Proposed Model

In Fig. 2 is sketched the essentials of a model, basically the 'Dobson–Brewer' circulation with a limitation in height, which meets all the above requirements. The rising air at the equator does not reach great heights, but turns polewards almost immediately. The higher branches of this circulation reach heights of only about 70,000–80,000 ft. It is postulated that the meridional circulation is quite fast just above the tropopause, this air taking not more than two months to reach temperate zones, while the upper flow is very much slower. This slow-flowing air, therefore, stays in the ozone-producing layers for periods of about a year and slowly feeds air enriched with ozone into the polar night, where it becomes available for transfer into the lower temperate stratosphere by the 'Dobson' mechanism.

The stratosphere above about 80,000 ft. is considered to be stagnant as regards large-scale meridional and vertical air movements, and a region where small-scale turbulent diffusion may be the important means of meridional transfer. This stagnant region is moist and since the humidity gradient is probably steep in the region of the transition to the circulating air, turbulent vertical mixing must be very small, otherwise higher humidities would be observed at lower altitudes.

It is evident that some water must be lost from this stagnant region by the limited vertical mixing and during the subsidence of air from the cold pool over the winter pole. It is tentatively suggested[8] that the summer polar regions may be the sources of replacement for this water. The possibility that thermonuclear explosions themselves are the main cause of the high humidity of the middle stratosphere is considered to be unlikely. It would mean that something in the order of 4×10^9 tons of water, depending on the lateral extent of the moist layer, were lifted to these heights during the explosions. An expenditure in energy of 2,000 megatons trinitrotoluene equivalent is required to evaporate this quantity of water, that is, more than an order of magnitude greater than the published[17] energy release of all nuclear bursts over water (64 megatons equivalent).

Note added in proof. The suggestion that rising moist air over the summer polar regions may be the source of the high humidities in the middle stratosphere now appears to be doubtful. Measurements made this summer by the Meteorological Research Flight (W. T. Roach, private communication) of the frost point of the air at about 45,000 ft. at lat. 80° N., showed the air there to be dry. In view of the dry air found at all latitudes at heights of about 50,000 ft., it is difficult to see where, on the Earth's surface, the moist air of the middle stratosphere can originate. The possibility of an extra-terrestrial source, such as a high flux of low-energy protons from the Sun followed by oxidization must be given serious consideration (C. M. de Turville, *Nature*, 190, 156 (1961)).

Discussion

This model is now shown to account satisfactorily for all the broad features concerning stratospheric circulation listed above.

(*a*) (i) *Tungsten*-185: The tungsten-185 data[9] are quite consistent with the circulation pattern outlined in this article. The bulk of the tungsten-185 is in the stagnant regions, the maximum concentration therefore remaining at the injection latitude. The lower fast circulation has been provided with tungsten-185 by possible gravitational settling of the larger particles or by some turbulent mixing from above and by that part of the injection put directly into the lowest part of the equatorial stratosphere.

(ii) *Strontium*-90: The vertical distribution of strontium-90, which showed a maximum concentration above 70,000 ft. at all latitudes in 1959[9], is explained because this distribution is the result of numerous injections, since 1952, made at several northern latitudes. That part of the debris placed in the stagnant stratosphere has been spread laterally by the slow diffusion processes. The strontium-90 debris injected between the tropopause and the lower limit of the stagnant stratosphere has been carried to high latitudes by the circulation and so was in a position to transfer to the troposphere comparatively quickly.

(iii) *Carbon*-14 : The world-wide distribution in 1955 of artificially produced carbon-14 (ref. 18) similarly showed a maximum concentration above 80,000 ft. at all latitudes, with small concentrations in the lower equatorial stratosphere. This again is in full agreement with the model shown in Fig. 2.

(iv) *Ozone*: Ozone is produced naturally by photochemical action, mainly in the middle stratosphere. Sinking air in the lower stratosphere is therefore generally rich in ozone while rising air is deficient. The low ozone concentrations in the lower equatorial stratosphere[19,20] indicate rising air there. Similarly, the high concentrations in the lower temperate stratosphere during the late winter or spring[19,20] are indicative of subsiding air. The limited circulation model, like that of Dobson and Brewer, is in full agreement with these general conclusions.

(*b*) Following the subsidence of moist air from high over the winter pole during the late winter and spring, higher humidities would be expected in the lower temperate stratosphere, together with the higher ozone concentrations which account for the spring peak in the total ozone. The Meteorological Research Flight humidity measurements at 48,000 ft. over southern England do not appear, at first sight, to be in agreement with this requirement. However, it must be remembered that the aircraft frost-point hygrometer is probably not accurate to better than 2° C. when measuring frost-points of about − 80° C. There is also some evidence that it may read systematically low[21]. A study of the actual measured frost-points reported at 48,000 ft. over England[4,5] show that the humidity mixing ratio was generally 0·0015 gm./kgm., while on more than 10 per cent of occasions it was more than 0·003 gm./kgm. If a value of 0·0015 gm./kgm. were taken as the humidity mixing ratio of the air reaching the lower temperate stratosphere via the lower fast direct flow, and if 0·04 gm./kgm. as found on the balloon flights[8] were taken for that arriving from altitudes above 80,000 ft. via the polar regions, then for the actual humidity in the temperate lower stratosphere to remain within the range of uncertainty of the aircraft frost-point hygrometer, at a value of 0·003 gm./kgm., say, only about one-twentieth of the air in the lower temperate stratosphere can have come from high altitudes.

A similar calculation for the ozone concentration using the mean data on the vertical distribution given by Ramanathan and Kulkarni[19] is in good agreement with this conclusion. A value of $4{\cdot}5 \times 10^{-7}$ gm./gm.

is taken for the ozone concentration of the low direct flow, 9×10^{-6} gm./gm. is taken for that pertaining to the air at 90,000 ft., while that at 50,000 ft. over middle latitudes during the spring peak is 9×10^{-7} gm./gm. These values lead to a conclusion that one-eighteenth of the air in the latter region can have originated at 90,000 ft.

(*c*) The tritium concentrations within the middle stratosphere over England[8] show that the transfer of air from these heights to lower altitudes is slow. The replacement of tritium by lateral mixing has been at least as great as that lost by vertical transport since the termination of weapon testing in 1958.

(*d*) The explanation by Stewart *et al.*[7] of the peaks in the fall-out of radioactive material of stratospheric origin reaching the ground in temperate and polar regions[7,22,23] is still valid. The circulation has fed debris from the lower equatorial stratosphere to that of temperate latitudes where conditions are more favourable for air transfer from stratosphere to troposphere. Staley[24] has shown that conditions favourable for this transfer are not uncommon at latitudes greater than 30°. Aircraft measurements[25] of the humidity and ozone distribution in the region of discontinuities in the tropopause have demonstrated that the transfer of air from stratosphere to troposphere is particularly strong in these regions. Transport along the isentropic surfaces often passing through such tropopause gaps is probable[26].

(*e*) The smaller quantity of fall-out in the southern hemisphere[22,23] can be explained by the fact that no significant injections of nuclear debris have been made south of the equator. The material placed in the lower northern equatorial stratosphere has been quickly transferred by the circulation to higher latitudes to the north. However, that debris produced in the stagnant stratosphere would be expected to diffuse to the south as well as the north. The debris at these heights can then feed the upper arms of the circulating air of the southern hemisphere and accounts for the comparatively small amounts that have fallen there.

The spring peak in the radioactive fall-out reaching the ground[27,28] and the total ozone[6] in extratropical regions are still considered to be the result of the subsidence of air from high over the winter pole. The sudden breakdown of the winter polar vortex may be associated with the onset of this subsidence[29].

(*f*) In August 1958, rhodium-102 was placed directly into the high equatorial stratosphere at heights of more than 100,000 and 250,000 ft. in rocket detonations. This rhodium-102 first became apparent[15] in ground air at Argonne, United States (42° N.), some thirteen months after its production in the upper atmosphere. This contrasts with a delay of 1–2 months[15,16] found for tungsten-185 injected lower in the equatorial stratosphere.

Although the presence of rhodium-102 is so far unsupported by other observers, this type of behaviour for high-altitude equatorial debris, compared with that from the lower stratosphere, is to be expected on the limited circulation model. The tungsten-185 debris is in the lower fast stratospheric circulation and so is transferred to middle latitudes quickly. The onset of the rhodium-102 in the ground air occurred in September. This suggests that subsiding polar air was not the source of this rhodium-102, but that the meridionally circulating flow was the mode of transfer to the lower temperate stratosphere. The upper arms of the circulation could have received the rhodium-102 by gravitational settling and by the limited turbulent vertical mixing from above. A steady increase in the rhodium-102 for the six months following its first detection in ground air suggests that the upper circulating air may already be sinking at these latitudes.

The difference of about one year in the time required for material from the two equatorial sources to reach temperate zones indicates the order of the difference in speed between the upper and lower arms of the circulation pattern.

The work of Burton and Stewart[29] on the concentration of natural radium *D* and radium *F* in the troposphere and lower stratosphere indicates a mean circulation from low to temperate latitudes in the lower stratosphere of about six months. This agrees well with the evidence in favour of a few months for the lower flow and about a year for the higher circulation.

(*g*) The most recent estimate of less than one year[32] for the stratospheric half-residence time is considered to refer to the mean life of the debris placed in the lower equatorial stratosphere. The shorter residence time[27,31,32] for material injected at high latitudes is not surprising, since when these injections are in the lower stratosphere, they simply by-pass the meridional circulation and are in regions favourable for air transfer from stratosphere to troposphere.

Equatorial debris above 80,000 ft. will have a much longer residence time and it may be that Libby's original estimate[33] of 10 ± 5 yr. applies to this material.

It might be expected that debris resulting from high-altitude events at high latitudes would have a shorter residence time than the equivalent equatorial debris, since it is produced directly in or near those regions scavenged annually by the 'Dobson' mechanism. Its actual residence time, however, will depend on the amount of mixing taking place toward the equator in the stagnant regions of the stratosphere over polar areas.

Concluding Remarks

Murgatroyd and Singleton[34] have calculated meridional and vertical velocities in the stratosphere by considering the transfer of heat from the radiational sources to the sinks in the stratosphere. Their findings are not inconsistent with the limited circulation pattern presented here. They do indicate that above 90,000 ft., in spring and summer, there is often a region with no meridional or vertical flow, while in winter there is a slow descending circulation toward the poles. The velocities given for this meridional flow show a decrease with increasing height in the lower stratosphere. This work also shows the presence, above the stagnant region, of very strong circulations.

It is not considered that the model sketched in Fig. 2 is invariable. It may be that the height of transition between meridional circulating to meridional stagnant air is variable in time and latitude. It is possible that the spring peaks are associated with a change in the height of this boundary in the polar regions. Measurements of the humidity in the lower stratosphere directly over the poles would be of considerable interest.

It would seem that the equatorial stratosphere does act as an efficient storage reservoir of radioactive material injected there during the course of weapon testing, if those injections are made at heights greater than about 80,000 ft. Lower than this, the

stratospheric circulation is very effective at transferring material from equatorial to temperate latitudes. It may be that short-lived fission products from medium-sized events at these heights add more to the fall-out reaching the ground than those from much larger injections at greater heights.

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ORIGIN OF THE BRITISH INSULAR RACES OF SMALL MAMMALS AND OF THE 'LUSITANIAN' FAUNA

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IN the early years of this century, Hinton and Barrett-Hamilton[1] put forward a theory which asserted that the populations of Microtine rodents in the Orkney, Hebridean and Channel Islands were relict forms, once present throughout mainland Britain in post-glacial times but displaced by later arrivals from the south. These island populations were believed to be most closely related to certain forms found in the Pyrenees, Alps and in Scandinavia, which were also considered to be relict. The principal evidence supporting this theory was the morphological resemblance between the island and alpine populations, and the presence of similar forms in Late Pleistocene deposits in southern England.

A re-assessment of the problem in the light of extensive new data, both geological and zoological, suggests that the majority of these island populations are not relict, but must be attributed to introduction by human agency. The relevant geological facts, now established independently of zoological evidence[2], are that at the maximum of the last glacial phase the ice extended as far south as Norfolk and South Wales at sea-level; the rest of Britain south of the ice consisted of tundra; and the maximum eustatic lowering of the sea-level was about 70–100 metres, that is, 35-50 fathoms. As an actual depression of the shore-line, this only applies to the unglaciated south. In the north it was accompanied by a very considerable isostatic depression of the land surface so that the depression of the shore-line was very much less; and it is therefore almost certain that the Shetlands, Orkneys and Outer Hebrides have never been connected to the mainland during or since the last glacial phase.

The fossil deposits said to support the relict theory (from fissure deposits at Ightham in Kent) are now seen to represent a very long period of interstadial and late glacial time, and provide no evidence of the presence of the 'relict' types, *Microtus corneri* Hinton and *Clethrionomys kennardi* Hinton, in the post-glacial colonization.

Extensive measurements of island and mainland voles, to be published separately, have now shown that the basic characters distinguishing the island forms are very few and merely represent extremes in the ranges of variation found on the mainland. These data do suggest the presence of a relict form of *Microtus*, but not of *Clethrionomys*, on the mainland. The *Microtus* from the north-west Highlands are believed to represent a relict population, of which the distinctive features (large size and complex first upper molars) have been replaced farther south to a variable extent by interbreeding with a later arrival (*M.a.hirtus* of the relict theory). The principal dividing line seems to be the Great Glen.

Theory of Human Introduction

In the case of those islands separated from the mainland by deep channels, the geological evidence alone is sufficient to show that all the mammalian species must have been introduced; but in order to distinguish between indigenous and introduced species on the more inshore islands, we must consider also the order in which the species colonized the country, and their chances of subsequent extinction on an island. The three rodent species, *Microtus agrestis*, *Clethrionomys glareolus* and *Apodemus sylvaticus*, are

Table 1. Distribution of Small Mammals on the Islands Discussed in the Text

	Microtus arvalis	*Microtus agrestis*	*Clethrionomys glareolus*	*Apodemus sylvaticus*	*Sorex araneus*	*Sorex minutus*	*Crocidura suaveolens*	*Crocidura russula*
Shetlands				+				
Orkneys	+			+		+		
Lewis*, Barra*, Man, Ireland				+		+		
Uist*, Eigg†, Muck†		+		+		+		
Raasay†, Skomer			+	+	+	+		
Mull†, Bute†		+	+	+	+	+		
Skye†, Islay†, Jura†, Gigha†, Arran†		+		+	+	+		
Scillies				+			+	
Jersey			+	+	+		+	
Guernsey	+			+				+

* Outer Hebrides. † Inner Hebrides.

characteristic of open grassland, scrub and closed woodland respectively. This distribution is related to their requirements of food and cover, which in turn are controlled by potential competition with each other. In northern Europe, *M. agrestis* alone occurs on the open tundra, *C. glareolus* appearing farther south, and *A. sylvaticus* yet farther south in the forest zone, whereas in Britain a similar sequence occurs in the transition from high to low altitude. It is therefore reasonable to assume that these three species would advance in the order quoted above at the time of the post-glacial colonization, with *M. agrestis* well ahead of the other species.

Many islands lack one or more of these species (Table 1). Because of their distribution by habitat, extinction due to climatic deterioration would affect *Apodemus* first and *Microtus* last. The chances of *Microtus* alone becoming extinct on any but the smallest islands are very small. The islands with which we are concerned are mostly large; for example, Lewis (870 sq. miles) and Raasay (25 sq. miles). Further, many of these islands lack the ground predators such as fox, cat, *Mustela* spp. and the adder *Vipera berus*, which are probably important enemies of *Microtus* and could conceivably cause extinction if the population was already very low.

Thus it can be postulated that the absence of *Microtus agrestis* from any large island indicates isolation since a time when the climate was severely arctic, and that any members of the fauna of the island which are characteristic of a more temperate climate must have reached the island after separation.

It is generally agreed that mice, including *Apodemus*, have been introduced by human agency; for example, to St. Kilda and Iceland. Trade among the British islands has taken place at least since mesolithic times and has involved the carriage of cattle and sheep (with accompanying fodder), building stone, timber, peat and roofing turf, all of which could have harboured voles and shrews as well as mice. Such trade is more extensive to small islands, which are deficient in natural resources, than to large ones like Ireland.

The difference of environment between mainland and island is often slight in terms of physical and botanical factors, but is vast in terms of mammalian neighbours; there are about 33 species of indigenous terrestrial mammals in northern France, 25 on the English mainland, and none on most of the islands. In particular, large size, the chief characteristic of most island forms, could have evolved rapidly due to the absence of ground predators. It is likely that the size of any species of small mammal is controlled by the interplay of opposing selective forces, intra-specific selection favouring large size, the presence of ground predators favouring small size, that is, the ability to escape into holes inaccessible to the predator. Rapid evolution would also be facilitated by the growth of a population from perhaps a single litter, allowing great possibilities of random divergence in the early stages of colonization. Examples of rapid evolution after undoubted introduction are provided by *Apodemus sylvaticus* on St. Kilda, and *Mus musculus* on North Bull Island in Dublin Bay, where a distinctive colour variety arose within a century[3].

Origin of the Individual Races

Microtus arvalis. This species is confined to Orkney and Guernsey, being absent from all the numerous western islands that are no more isolated, but are yet without *M. agrestis*. In fact there is no evidence, apart from these two cases, that it has ever occurred in the British Isles in post-glacial time. Orkney is separated from the mainland by a channel of 30–40 fathoms and its fauna as a whole suggests very early isolation. For example, there are no stoats, *Mustela erminea*. This species is widespread in the Arctic and is unlikely to have lagged behind its prey during the colonization, but equally unlikely to have become extinct on six separate islands in the continued presence of its prey. The *Microtus* themselves are absent from four large islands which are no more isolated and not separated by deeper channels than the others. Introduction from the continent of Europe could have taken place at any time since the Viking era when trade with Denmark, for example, was very extensive. Establishment would be easier in Orkney than in mainland Britain, because of the absence of competitors and ground predators.

The situation on Guernsey is similar. The absence of *M. arvalis* from all the other Channel Islands is difficult to explain on the relict theory since there is no evidence that they were isolated before Guernsey.

Microtus agrestis. The *Microtus* on the Outer Hebrides are clearly introduced since the channel separating the group from the mainland is everywhere deeper than 50 fathoms, which alone precludes contact under post-glacial conditions. Study of *M. agrestis* from the north-west Highlands of Scotland, that is, from north-west of the Great Glen, shows them to possess the complex variant of M^1 almost as universally as do the Outer Hebridean populations. It therefore seems likely that the latter have been introduced from the north-west coast.

The *Microtus* of the Inner Hebrides can be divided clearly into two groups having respectively a complex and a simple M^1 as follows: 'complex'—Skye, Eigg, Islay; 'simple'—Muck, Mull, Gigha, Arran. Those from Skye can be considered endemic since the channel is less than five fathoms deep and the fauna as a whole shows no impoverishment when compared with that

of the mainland. Eigg is much more isolated (by 30–40 fathoms) with an impoverished fauna and therefore it is likely that the *Microtus* have been introduced (from the north-west Highlands). Islay, through Jura, is separated from the mainland (south of the Great Glen) by a shallow channel (5–10 fathoms) and therefore the *Microtus* population is likely to be indigenous. If so, being complex, it must be considered to be truly relict, that is, isolated before the arrival of the 'hirtus' characters. This places it in the same category as the north-west Highland form, except that in this case the isolation is complete instead of only partial. This interpretation is supported by the fact that the complex condition is more extreme and general than in any other population examined.

Muck is well isolated (30–40 fathoms) and M^1 is simple. It therefore seems likely that introduction has taken place either from the southern part of the mainland or from one of the more southern islands (for example, Mull). The *Microtus* of Mull have M^1 almost 100 per cent simple. The island is connected to the mainland on the south side of the Great Glen (through Lismore Island) by a submarine ridge 10–20 fathoms deep. It is therefore conceivable that it could have been populated before separation; but if that had been the case one would expect the population to be complex, as in the case of Islay. The most likely explanation therefore is that the *Microtus* have been introduced accidentally from the southern part of the mainland coast. The remaining islands of Gigha and Arran are probably in the same category. Arran in particular is well isolated (by 20–30 fathoms).

Microtus seem to be absent from the remaining large islands of the Inner Hebrides, namely, Raasay, Rhum, Tiree, Coll and Colonsay, some of which are no more isolated than Mull. To summarize, accidental introduction is postulated for five Inner Hebridean islands: Eigg, Muck, Mull, Gigha and Arran.

Clethrionomys glareolus. The islands of Raasay, Skomer and Jersey are similar and at the same time unique in having a large form of *Clethrionomys* with complex dentition (M^3) and no *Microtus*. The main argument in favour of accidental introduction is that *Clethrionomys* is unlikely to have colonized the country ahead of *Microtus*, but the latter is equally unlikely to have become extinct on the islands while *Clethrionomys* survived, especially in such marginal habitats to which *Microtus* is particularly well adapted. Absence of *Microtus* would favour adaptation to a grassland habitat, in particular adaptation to the tougher but more reliable and abundant food of *Microtus*. The complex dentition of these races represents a condition which is present, but in a smaller proportion of individuals, on the mainland, and the change in incidence of the complex condition could have taken place almost in one step if the colonizing parents happened to be complex. Such an occurrence in an isolated habitat on the mainland has already been recorded[4]. Once the *Clethrionomys* were established in all available habitats, subsequent colonization by *Microtus* would be considerably more difficult. The limitation of this type of *Clethrionomys* to these few islands is explained on the present theory by assuming that these were the islands on which *Clethrionomys* happened to have been introduced ahead of *Microtus*; it is inexplicable by the relict theory.

The *Clethrionomys* on Mull have not developed a complex dentition presumably because they were confined to their normal ecological niche by the prior presence of *Microtus*. If it is accepted that the latter have been accidentally introduced, as argued above, then the same must be said of the *Clethrionomys*.

Apodemus sylvaticus. *Apodemus* is more likely than the voles to be transported accidentally and at the same time is likely to have been a late, woodland colonizer. Its survival on small treeless islands does not contradict this, since there it is free from competition from voles. It is therefore postulated that all the following populations of *Apodemus* have arisen by accidental introduction: Shetland, Orkney, Outer Hebrides, all Inner Hebrides except Bute, Ireland, Isle of Man, Scillies and Channel Islands.

Sorex minutus. This species is very widespread on the islands and seems likely to be indigenous on many of them. This is not unexpected since its present range, extending to an altitude of more than 4,000 ft. in Scotland, suggests that it may well have been in the forefront of the post-glacial colonization. Introduction need only be postulated for Orkney, the Outer Hebrides and perhaps some of the more isolated Inner Hebrides. The presence of *S. minutus* in Ireland could be explained by early post-glacial entry from Scotland, but survival in the south of Ireland during the last glacial phase cannot be ruled out, nor can introduction.

Sorex araneus. From its distribution and present habitat this species appears to have been slightly in advance of *M. agrestis* in its post-glacial spread. Most of the small islands on which it occurs are not deeply separated from the mainland and therefore on these it may be indigenous (Raasay, Skye, Mull, Islay, Jura, Gigha, Bute, Skomer). It is only present on a very small proportion of the more isolated islands (Arran, Jersey) on which it was probably introduced.

Crocidura spp. This genus is present only on the Scilly and Channel Islands. It has a much more southern distribution than any of the others dealt with and is thus likely to have been a late colonizer. It is therefore considered to have been introduced on all these islands. The ubiquity of *C. suaveolens* on the Scilly group has been used as an argument against chance introduction[5]. But its usual habitat, among the kelp on the storm beaches, coupled with the extensive use made of the kelp for manure and reduction to ash, suggest clearly how it could have been distributed among the islands.

Origin of the Irish Fauna

The so-called 'Lusitanian element' in south-west Ireland has been a stumbling-block to all students of the history of the British fauna and flora. Recent authors have been forced to postulate glacial survival on land now submerged off south-west Ireland[6], or very early post-glacial colonization by land[7]. We may ask first why a land link should have allowed passage of such a non-athletic creature as the slug *Geomalacus maculosus*, which typifies the Lusitanian element, and not of such vertebrates as rodents and reptiles which abound in Iberia? Alternatively, it is inconceivable that the vertebrates but not the invertebrates should have perished in a glacial re-advance. The present distribution of the Lusitanian species, confined to the warmest corner of Ireland, scarcely suggests great tolerance of peri-glacial conditions. On the other hand, trade between Iberia and Ireland has taken place since mesolithic times, and the size

and habits of the Lusitanian species predispose them to such a form of transport.

The Lusitanian flora has probably had a similar origin, but in this case there is the additional possibility of carriage by birds which could have taken place at any time in the post-glacial period, Ireland and Iberia lying on a major migration route. In a recent survey of the botanical problem, Godwin[8], who dismisses glacial survival but accepts the idea of early post-glacial colonization by land, lists only one Lusitanian species from an early post-glacial horizon, namely, *Erica mackaiana* from the Atlantic period. This is a species which could easily have been carried by birds.

The absence of some of these species from the climatically similar south-west of England is to be expected, since there they would meet competition from related, indigenous species which are absent from Ireland (or have only been introduced later from England).

The Irish fauna as a whole is of such an erratic composition as to suggest a series of chance introductions rather than an orderly sequence of arctic, followed by more temperate, faunas, until at some stage the supply has suddenly stopped due to severance of the land connexion. The absence of voles suggests that the last connexion (to Scotland) was severed at a very early climatic stage. However, a narrow channel or wide river would effectively bar small mammals while allowing unrestricted passage to many insects, birds and plants. Thus a south-flowing river could have allowed such selective colonization in the southern Irish Sea region while a truly terrestrial bridge in the north was inaccessible to most species because of the climatic severity.

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THE DEVELOPMENT OF THE NEON GLOW LAMP (1911–61)

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FOR ten years after its discovery (1898), the world's supply of pure neon was contained in two small tubes inverted over mercury which stood on Sir William Ramsay's mantelpiece, and experiments on its use as a commercial illuminant were scarcely to be expected. In 1908, however, Georges Claude designed an extra stage for his oxygen plant and produced, hourly, 12 litres of a gas containing 45 per cent of mixed helium and neon. While purifying some of this gas in Ramsay's laboratory, I noticed the great ease with which neon conducted the electrical discharge, and, two years later, working in the Cavendish Laboratory, I found that the cathode fall with aluminium electrodes in pure neon was 200 V. This indicated that, provided conditions were such that there was no positive column, a lamp would operate at approximately this voltage. Further measurements of the spark-potential confirmed this, and several experimental lamps were made, some of which would start with 150 V. and run at 85 V. The important feature of all of them was operation on the domestic voltage of 220, although in France and the United States, where the usual value was 110 V., they were not of great interest. A provisional patent was applied for in March 1911, but was not completed as I had to abandon this work on taking up an appointment abroad. These lamps, in which substantially the whole of the light comes from the negative glow, are known as 'glow lamps', or familiarly in recent years 'neons', and a useful property is that the glow follows the shape of the cathode and can thus be used for illuminated signs. In the long, tubular, high-voltage neon lights, the illumination comes from the positive column. These were first shown in public by Claude in 1910.

Experimental glow lamps were made by Dr. D. McF. Moore in the General Electric Laboratories, in Schenectady, from 1914 onwards, but even by 1920 no reliable lamp had been produced which worked on 110 V., and it was decided that this type of illuminant was of no great value. In Germany, on the other hand, the firm of Pintsch A.G. realized that although the efficiency of glow lamps, normally an important criterion, was low, there were many more uses than appeared at first sight. The smallest filament lamps for 220 V. then made were 15 candle-power, taking 20 W., and these were very expensive and fragile; in many cases, such as for nightlights and in mines, they could be replaced advantageously by 3–5 W. glow lamps. These were also valuable as indicators on switchboards. In consequence, their research department, under Dr. F. Schröter, undertook extensive investigations during the War, and in 1918 produced the first commercial glow lamps. These resembled filament lamps in size and were suitable for 220 V. In 1920 Osram G.m.b.H were making similar lamps designed by Dr. F. Skaupy at the rate of 500 a day.

It must be remembered that at that time very little was known about the mechanism of the discharge and that practically all the experimental work was empirical. The early lamps suffered from sputtering of the electrodes, which blackened the tube and absorbed the gas, sometimes in a few hours, while the methods of evacuation and removal of gases from the walls of the tube would now be considered crude. Troubles due to these causes had been met with and largely overcome by Claude and other makers of high-voltage tubes, but their remedies were not all suitable for the production of small lamps in quantity, and much new work became necessary. Freedom from diatomic gases had always been considered essential for preserving the colour of the discharge and obtaining the minimum spark-potential until Pintsch A.G. in 1917 showed that very small quantities of these gases not only did not affect the colour but also

lowered the spark potential and reduced sputtering to a quite unexpected degree. Shortly afterwards, N.V. Philips recommended a helium–neon mixture containing up to 5 per cent of argon for the reduction of sputtering, and in 1926, T. E. Foulke at Schenectady, with far more theoretical knowledge at his disposal and after prolonged investigations, confirmed the value of a mixture of this nature.

Meanwhile, efforts were being made to find materials with a low cathode fall which would enable glow lamps to start and run on 110 V. d.c. Skaupy solved this problem in 1917 by coating metallic electrodes with barium azide and afterwards heating them, but simplification and improvement of the process took nearly five years. N.V. Philips in 1921 made tubes with cathodes of magnesium and beryllium alloys which would start below 110 V. and obtained improved results by introducing potassium nitrate. These results aroused great interest in the United States, where Foulke had been studying the effect of gas composition, as mentioned above. He soon succeeded in producing a tube with magnesium electrodes which would start with 70 V., and three years later he had developed two new coating processes now known as the 'oxide' and the 'azide'. The first started with barium carbonate, which was heated in the tube to 1,000° C. or more and activated by a discharge with a steep-fronted wave-form. The second was similar to Skaupy's but a little cæsium was added to the barium and the subsequent processes were strictly controlled. The result was a lamp starting with 45 V. a.c.

During this work, an outstanding design of lamp was evolved. The lamps, named *T*-lamps, were made from tubing ¼-in. or less in diameter, with two parallel wires as electrodes, the overall length being less than 1 in. These miniature lamps, bright enough to be seen as indicators and readily incorporated in instruments, caused an increased demand, but it was not until about 12 years ago in the United States and a few years later in Britain that a remarkable development took place, caused by the introduction of glow lamps into domestic appliances such as electric irons and refrigerators, and later into switches. The demand was far greater than had been foreseen and much ingenuity had to be exercised in developing machinery to carry out what was still a highly complicated process requiring the greatest precision. It is estimated that the world output of neon glow lamps is now about 80 million a year, and is increasing rapidly. Quite 80 per cent of these are of the *T* type. These can be sold for less than a shilling, are remarkably robust and reliable, and will run for their rated life of 25,000 hr. at a cost of twopence.

Within the past few years a demand has arisen for 'high brightness' lamps which can be seen readily in full daylight. This could not be met by minor modifications of existing processes and required a great deal of additional research, one item being a study of lanthanum and its alloys. Satisfactory lamps are now in production in several countries, but research is by no means at an end.

These notes are extracted from a longer account of the development which I have compiled, and it may be mentioned that a very valuable publication has just appeared dealing with the scientific basis of the glow discharge, with 156 references, written by M. P. Lemaigre-Voreaux as a D. ès Sc. thesis presented to the University of Lyons.

OBITUARIES

The Right Hon. the Lord McGowan, K.B.E.

Lord McGowan of Ardeer, who died on July 13, was honorary president of Imperial Chemical Industries, Ltd., of which he was chairman from 1930 until the end of 1950 and for the creation of which, with Sir Alfred Mond, afterwards Lord Melchett, he was mainly responsible.

Henry Duncan McGowan was born in Glasgow on June 3, 1874, and educated at Allan Glen's School, entering the Glasgow offices of the Nobel's Explosives Co. in 1889 as a junior clerk or office boy. He became assistant successively to the home and to the overseas sales manager, and then, in 1902, deputy assistant sales manager, and in 1909 assistant manager. In December 1915 he was appointed to the board and became primarily responsible for co-ordinating the Company's activities in relation to the requirements of the Ministry of Munitions. In 1918 he was appointed managing director, and in recognition of his services during the War he was created K.B.E. At the conclusion of the War he was responsible for the merger of all the important explosives and ammunition companies in Britain into Explosives Trades, Ltd., of which he became chairman and managing director. Earlier, in 1910, he had used the Hamilton Powder Co. as a nucleus for the amalgamation with three other explosives companies and the Dominion Cartridge Company of Quebec to form Canadian Explosives, Ltd., and in the winter of 1923–24 he also negotiated the formation of African Explosives and Industries, Ltd., in South Africa.

The creation in 1926 of Imperial Chemical Industries, Ltd., by the joint efforts of McGowan and Sir Alfred Mond, out of Nobel Industries, Ltd., Brunner Mond and Co., Ltd., the United Alkali Co., Ltd., and the British Dyestuffs, Ltd., was thus the crowning of his persistent efforts towards amalgamation and rationalization of the chemical industry of Britain, and McGowan became president and Mond chairman of the new Company. Mond (afterwards Lord Melchett), however, died in 1930, and McGowan succeeded him as chairman, and the growth and shaping of the enterprise were very largely his work. By 1938, however, he realized that it had become too large to be managed efficiently from the centre and that the advantages of large-scale production were in danger of being offset by rigidity, and McGowan initiated some degree of decentralization.

By temperament McGowan was something of an autocrat, but he was a good mixer and, working hard and thoroughly himself, he expected the same of others. What mattered was the way the job was done, and with marked ability to grasp the essentials of a problem, he was also swift to see and seize an opportunity. Melchett had been elected president of the Society of Chemical Industry in 1930 in view of the Society's jubilee meeting in London in the following year. On Melchett's death McGowan took his place, and his presidential address to the Society

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on "Science and Industry" in July 1931, and his Messel Memorial Lecture to the same Society at Cardiff three years later on "The Uneven Front of Research" display very fairly his outlook on science and technology.

He was created a baron in 1937 and received honorary degrees from the Universities of Glasgow (1934), Birmingham, Oxford, Durham and St. Andrews. He married in 1903, Jean Boyle Young, of Paisley, who died in 1952, and he is survived by two sons and two daughters, the title passing to his elder son, Harry Wilson McGowan.

R. Brightman

Sir Hugh Warren

Henry William Hugh Warren was born on June 30, 1891, and died on June 18, 1961. He is survived by Lady Warren and a daughter. An only son was killed serving with the R.A.F. in 1939.

Hugh Warren, in 1929, took over the Research Laboratory at the British Thomson-Houston Co., Ltd., Rugby, which, six years previously, he had helped the late R. C. Clinker to start. In 1938 he was appointed to the board of B.T.H. as director of research. He remained in charge of the Laboratory until 1945, becoming managing director of B.T.H. in that year and managing director of A.E.I., Ltd., in 1948, retiring in 1954. He received honorary degrees from the University of Bristol in 1942 and from Birmingham in 1946, and was knighted in 1951.

It is as leader of what is now the A.E.I. Research Laboratory, Rugby, a job he did for sixteen years, that 'H. W.' will be remembered. He was alert and interested in everything, intensely energetic and always desirous of presenting the work of his Laboratory in as impressive a manner as possible. His own subject was insulations. It was in 1916, on the then vital matter of the insulation of magnetos, that he showed the energy and enthusiasm to engage in, and pursue relentlessly, difficult technological problems that proved to be characteristic of the man. He directed with vigour the pioneer work of his Laboratory on sound reproduction, talking films and discharge lamps. During the War, starting with the *VFO* ('Valve for Oliphant') klystron, he built up a large effort on centimetric radar, including work on magnetrons and other microwave valves, radar sets and the silicon and germanium crystal valves which were the forerunners of the modern semiconductor devices.

He played a part of considerable significance in bringing about cordial and fruitful co-operation between teams of scientists from industry and those from Government and military establishments that frequently met together at Rugby to attempt to solve some of the problems of the times. Such meetings were memorable and, whether 'H. W.' took the chair or not, his presence with its sense of urgency and promise of achievement helped us all to accomplish things that seemed to border on the impossible. He enjoyed overcoming the difficulties of people as well as those of physics and production.

Hugh Warren was a member of many engineering institutions, councils and committees, and was the author of many technical papers and a comprehensive volume published in 1931 entitled *Electrical Insulating Materials*.

He loved writing, and would spend odd minutes as well as long hours on semi-technical essays, some of which were gathered together in a volume called *ON—Some Irresponsible Jottings, Scientific and Otherwise*, published for private circulation in 1944. He had a great sense of humour and was a pleasant companion and a kindly and courteous leader; he will be remembered with admiration and affection.

L. J. Davies

NEWS and VIEWS

Technical Managing Director of Reactor Centrum Nederland : J. Pelser

Johannes Pelser has been appointed technical managing director of the Reactor Centrum Nederland foundation in succession to J. J. van Rijsinge. Johannes Pelser was born at Dordrecht on March 21, 1925, and matriculated at the Christian Grammar School at Dordrecht; afterwards he studied at the Technical University at Delft, where he obtained his certificate for physical engineering in 1951. Following this he joined the Foundation for Fundamental Research on Matter, but was transferred to the Reactor Centrum Nederland foundation when it came into existence in 1955. While at the Foundation for Fundamental Research on Matter, he was seconded to the (Netherlands–Norwegian) Joint Establishment for Nuclear Energy Research at Kjeller, Norway. Here he worked for four years and took part in the research work with the nuclear reactor *Jeep*. A short time after the setting up of the Reactor Centrum Nederland, Pelser returned to the Netherlands and took an active part in the preparations for the building of a research centre at Petten. In 1957 he was sent to the United States as a representative of the Reactor Centrum Nederland to supervise the fabrication of the high-flux reactor. By the end of 1958 he was appointed head of the Reactor Department at Petten, taking charge of the preparation and experiments with, and the operation and maintenance of, not only the low-flux reactor but also the high-flux reactor.

Aircraft Propulsion at the College of Aeronautics, Cranfield : Prof. A. H. Lefebvre

Prof. A. H. Lefebvre has been appointed to the chair of aircraft propulsion at the College of Aeronautics, Cranfield, in succession to Prof. A. G. Smith, who is now head of the Mechanical Engineering Department, University of Nottingham (see *Nature*, 187, 743; 1960). After leaving Long Eaton Grammar School, Prof. Lefebvre served an apprenticeship at Ericssons Telephones, Ltd., where he was employed afterwards for several years on engineering production and inspection. He studied electrical engineering at Loughborough College and mechanical engineering at the University of Nottingham. He is a graduate of the University of London and a postgraduate student of the Imperial College of Science and Technology. Since 1952, Prof. Lefebvre has been with the Aero-Engine Division of Rolls-Royce, Ltd., where his main responsibilities have been in combustion research and in the design and performance analysis of gas turbine

combustion systems. He is the author or co-author of several published papers on combustion and heat transfer, and is the United Kingdom editor of the new review series, *Progress in Combustion and Propulsion Science and Technology*. Until recently he was a member of the Combustion and Propulsion Panel of the Advisory Group for Aeronautical Research and Development of NATO, in which capacity he contributed as author and editor to several publications of the Advisory Group for Aeronautical Research and Development.

Photography at the Federal Technical College, Zurich : Prof. W. F. Berg

Dr. W. F. Berg has been appointed professor of the Photographic Institute of the Federal Technical College in Zurich, in succession to Prof. J. Eggert. Dr. Berg has already established an outstanding position as an authority on scientific aspects of photography. He started research work at the early age of sixteen in the X-ray laboratories of Siemens in Berlin under his father, Otto Berg, and graduated Dr. phil. of Berlin in 1932. The activities of the Nazis brought him to the University of Manchester in 1933, where he studied the growth of crystals, for which he was awarded a Ph.D. degree in 1936. In the same year he joined the Research Laboratories of Kodak, Ltd., where he soon began work on the fundamental processes in the formation of the photographic latent image. This work, on the temperature coefficient of sensitivity and the time required for build-up of the latent image, gave results in complete agreement with the theory of Gurney and Mott that the formation of the latest image was a two-stage process, and had much to do with the general acceptance of that theory. This work was followed by other investigations of the photographic process, less spectacular in their impact maybe, but not less important. This work was recognized by the award of a D.Sc. (Manchester) degree. For about a dozen years Dr. Berg has been in charge of aspects of research into photographic emulsions of more immediate practical application. His present appointment is a not unnatural result, not only of his authoritative position in photographic research work but also of his interest in education. He has been interested for many years in education in medical photography and is a member of the Education Committee of the Institute of British Photographers.

Editor of *Discovery* : Mr. Gerald Leach

Gerald Adrian Leach was recently appointed to the editorship of *Discovery*. Born in Colombo in 1933, Mr. Leach was educated at Glengorse School and Dauntsey's. He read for the Natural Sciences Tripos at Clare College, Cambridge, taking Part 1 in 1954, in physics, mathematics, geology and mineralogy, and Part 2 in 1955, in geology. Since Cambridge, he has been a full-time science writer and producer. In 1957 he worked on the *Manchester Guardian* as a general reporter and assistant to the Science Correspondent, and the following year was script-writer for scientific documentary films with the Film Producers Guild. In 1959 he wrote and presented 35 programmes in the Associated Television science series, "It Can Happen To-morrow". In October that year he joined the BBC Television Outside Broadcasts Department as assistant producer on the 1960 "Eye on Research" television series. In 1960 and early this year he helped to plan, and write the scripts for, Dr. Bronowski's series, "Insight".

British Association for the Advancement of Science

The annual report for 1960–61 of the Council of the British Association to the General Committee characterizes the year as one of major expansion with a total audience of 140,000, or nearly double that of any previous year, and a rapid growth in the demand for lectures and other forms of presentation of science (Pp. 31. London: British Association for the Advancement of Science, 1961). Three new Area Committees were established during the year, making a total of sixteen, and the Junior British Association meetings continued to expand, while an entirely new type of meeting was the science fair held in Manchester in March 1961. A grant of £6,000 from the Leverhulme Trust Fund enabled a research project for the study of the generating of mathematical insights to be initiated under Dr. Z. P. Dienes, and the work of the Unit is to last two years. During the year, films have been shown to audiences totalling 78,000 and other visual aids to audiences totalling 10,000. Some twelve receptions were organized, at which science teachers were shown a programme of films and other visual aids display. A survey of the use of visual aids by science teachers in schools in Essex was reported in *The Advancement of Science* for September 1960 and a more detailed report of the survey was in subsequent issues of the *Film User*. Following the Cardiff meeting, eight research committees were appointed on the recommendations of various sections and grants were made to three of these.

Successful Operation of *Nimrod* Injector

On August 1 the first proton beam accelerated to the design energy of 15 MeV. was obtained from the linear-accelerator injector which forms the first stage of *Nimrod*, the 7,000-MeV. proton synchrotron under construction at the Rutherford High-Energy Laboratory of the National Institute for Research in Nuclear Science, Harwell. The linear accelerator consists of a cylindrical copper cavity approximately 44 ft. long and 5·5 ft. in diameter, with 48 drift tubes spaced along its axis, the whole being enclosed in a vacuum envelope. Approximately 1 MW. of radio-frequency power is supplied to the cavity at its resonant frequency of 115 Mc./s. to set up the required accelerating fields across the gaps between drift tubes. The function of the drift tubes is to screen the protons from the field when its direction reverses every alternate half-cycle, and they also contain focusing magnets which constrain the protons to form a proper beam close to the machine axis. The drift tubes increase in length along the tank to correspond with the increase in proton velocity. Protons enter the linear accelerator with an initial energy of 0·6 MeV. from a pre-injection stage which is basically a conventional d.c. accelerator. Pulsed 15-MeV. beams will be delivered by the injector to the synchrotron at rates up to 2 per sec. and at pulse lengths up to 2 m.sec. Within the synchrotron, they will be constrained to a circular path by a suitably varying magnetic field. In this way they will make many revolutions, receiving a small acceleration at a particular point in the orbit until the output energy of 7,000 MeV. is reached. Construction work on *Nimrod* started in August 1957 and is expected to be completed during 1962.

Aeronautical Research in Asia and Africa

The International Civil Aviation Organization is to participate with the Governments of India, Morocco,

Thailand and the United Arab Republic in setting up and operating an aeronautical research laboratory and three civil aviation training centres. The laboratory is to be at Bangalore, India, where an Aeronautics Department in the Indian Institute of Science already exists. The prime objective will be the investigation of flight problems related to the design, construction and operation of aircraft in India. The laboratory will also undertake research and development work in allied fields of importance in India's economy, such as wind power and industrial aerodynamics.

The three new civil aviation training centres are to be in Morocco, Thailand and Cairo (U.A.R.). All three aim to train personnel in aircraft maintenance and operation to the recognized International Civil Aviation Organization standard. These centres will be open to trainees of neighbouring countries. Thus, the Moroccan centre in association with a similar Tunisian establishment will serve other African countries; Thailand will cater for south-east Asia; and Cairo will be the centre for the Middle East. In addition, the International Civil Aviation Organization is to help the centre already operating in Mexico City to increase its capacity and raise the level of training. In this way it is hoped that the supply and standard of trained civil aviation personnel throughout the world may be satisfactorily increased and improved to meet the increasing demand from the still expanding field of air transport. The Governments concerned and the United Nations Special Fund will provide the necessary financial support. The International Civil Aviation Organization will administer the scheme and provide any necessary additional funds.

Sterilizing Materials for Hospitals

All new hospitals in the United Kingdom will include a central sterile supply department, and many existing hospitals are already planning or constructing such departments. A small book, edited by Brian Watkin, assistant editor of *The Nursing Times*, describes the experience which has already been gained in setting up central sterile supply departments (Pp. 58. London: Macmillan and Co., Ltd., 1961. 4*s*. 6*d*.). Such departments have not only produced considerable financial economies but also have enabled a limited supply of nurses to be used to the best advantage, as well as to reduce the incidence of hospital cross-infection. Unlike the United States, where each financially independent hospital has its own central sterile supply service, the grouping of hospitals in Britain under regional hospital boards means that the arrangements for central sterile supply departments may differ from group to group. This booklet describes some of the experiments which have taken place and will be of great value to those responsible for planning hospital services as well as the doctors and nurses who work in them.

The South African Museum

The annual report of the South African Museum for 1959–60 records the completion of plans for the extension of the main Museum (Pp. ii + 19 + 4 plates. Cape Town: South African Museum, 1961). It also refers to the low scale of museum salaries and the consequent difficulty in obtaining personnel capable of assembling educational exhibits and undertaking research. The Board is endeavouring to establish equality of museum and university scales of pay. Several field trips were undertaken by the staff, most of them with the definite policy of collecting material to fill gaps in the collections. Remarkable progress has been made in the re-organization of several of the exhibition galleries, especially in the "Hall of Man", where the European Stone Ages are well illustrated, and a striking exhibit clearly shows the importance of South African ape men. An innovation in South African museums was the installation of earphones, by which visitors may, on inserting a coin, hear the calls of some common birds and at the same time see the animals in the case.

Modern Methods of Traffic Control

An exhibition of modern traffic control arranged by the Westinghouse Brake and Signal Co., Ltd., will be on view at the Science Museum, South Kensington, during September 12–October 1, to coincide with the opening there, by Lord Brabazon of Tara, of the London conference of the International Commission for Transport Museums. It illustrates, by means of actual equipment and working models, a few of the types of device employed in controlling safely the more rapid flow of traffic on road and on rail. Particular attention is paid to those regions of British Railways undergoing electrification. The exhibition includes the 'Westronic' demonstration model, which shows how all the points and signals in a particular signalling location are controlled, some distance away, from a central control cabin, which also receives an indication of the condition of the remote points and signals and the position of trains passing through. There is also an automatic barrier machine and its associated equipment, which ensures that road traffic is stopped for the shortest possible time at level crossings. Other railway signalling equipment devised to prevent false operation or damage to it by interference from the traction system will be shown in model form. Among the road equipment is a road traffic-lane divider, for varying the width of traffic lanes at the approach to main junctions or cities according to the direction and volume of traffic.

British Regional Development Plan for Courses for the Diploma in Technology

A regional development plan for courses leading to the diploma in technology and the diploma in technology (engineering) has been drawn up by the Regional Advisory Council for Technological Education, London and the Home Counties, after consultation with industry, the Ministry of Education and the local education authorities in the area (*Regional Development Plan for Courses for the Diploma in Technology*. Pp. 16. London: Regional Advisory Council for Technological Education: London and Home Counties, 1961). It is the policy of the Council to encourage and build up such courses so as to secure maximum efficiency. The need for providing such courses in anticipation of the 1964 and 1965 period, when a major influx of eighteen- and nineteen-year-olds is expected, is kept in mind, as well as the requirements of industry. The development of 'end-on' as well as 'parallel' courses will be encouraged as one means of making the best possible use of available industrial training places, even though this may present problems of staffing and time-tabling in the technical colleges. Another method may be that of the 'thick' sandwich courses of at least three years full-time academic study, together with a minimum of one year of industrial training during the course, and the need for the continuing development of Higher National

Diploma courses will not be overlooked. Stress is laid on the need for flexibility in this development, of which a synopsis is included in the pamphlet, together with some notes on the particular courses.

The Harkness Fellowships of the Commonwealth Fund—New York

THIRTY Harkness fellowships of the Commonwealth Fund—New York are tenable during 1962 for education and travel in the continental United States. The fellowships are offered to candidates from the United Kingdom, Australia, New Zealand and Western Europe, who are British subjects and either are graduates or have experience in Government service, the professions, the arts, branches of business or industry or other comparable careers or vocations, and have the equivalent of a degree in qualifications conferred by professional bodies or an attested level of professional competence. Forms of application, which must be returned before November 15, can be obtained from the Warden, Harkness House, 38 Upper Brook Street, London, W.1, from whom further details can be obtained.

The Institute of Scientific Information

A BROCHURE describing the services, publications and techniques adopted by the Institute for Scientific Information (Philadelphia, Penn.) has now been issued by the Institute, to which Eugene Garfield, its director, contributes a foreword. He states that the primary purpose of the Institute is to increase the productivity of research through a programme of dissemination and retrieval of information, screening, coding and indexing systems, design, research and development. The brochure describes the scope of *Current Contents* and the *Index Chemicus*, the *Index Medicus* programme, the *Citation Index* project and the three projects still in development: the transliterating typewriter, the *National Newspaper of Science*, and the *National Directory of Science*.

Polish Textile Research

THE journal of the Politechnika Łodźka (*Zeszyty Naukowe Politechniki Lodzkiej*) is published in five sections, corresponding to chemistry, applied chemistry, electricity, mechanics, and textiles. Volumes 6 and 7 of the textile section have been issued, and provide some ten extensive papers on subjects ranging from the variable delivery of metering pumps to the cleaning of bast fibres by passing them through fluted rollers. The work is described in great detail (in Polish) with summaries in Polish, English and Russian. The reports are of high standard, and should be available in textile laboratories. Copies can be obtained from the Politechnika Łodźka, ul. Gdańska 155, Łodź.

Announcements

DR. FABIAN RECALDE, former director of the National Institute of Nutrition in Quito, Ecuador, has been appointed to the staff of the Food and Agriculture Organization as a nutrition officer. Dr. Recalde's task will be the co-ordination of field projects in Latin America that require joint action by the Organization and the United Nations Children's Fund. These projects chiefly concern improving material and child nutrition.

DR. E. L. POWERS, associate director, Division of Biological and Medical Research, Argonne National Laboratory, Illinois, will deliver the seventh Douglas Lea Memorial Lecture, entitled "New Considerations on Survival Curves and Target Theory", at a meeting of the Hospital Physicists' Association, at 5.15 p.m. on September 15, in the Chemistry Department, University of Leeds. Further information can be obtained from Dr. J. R. Mallard, Department of Physics, Hammersmith Hospital, Ducane Road, London, W.12.

THE fifth international conference on "Pigment Cell Biology" is being sponsored by the New York Academy of Sciences and will be held in New York City during October 11–14. The conference will deal with melanoma and closely related fields. Further information can be obtained from Vernon Riley, Sloan-Kettering Institute for Cancer Research, New York 21, N.Y.

THE fourteenth international instructional course on "Fine Measuring Instruments" is being held by Carl Zeiss, Jena, at the Imperial College of Science and Technology, London, during September 11–22. Further information can be obtained from Carl Zeiss Scientific Instruments, Ltd., 12*a* Golden Square, London, W.1.

THE newly formed American Society for Cell Biology is holding a meeting at the Edgewater Beach Hotel, Chicago, Illinois, during November 2–4. Three symposia are included in the programme, which will cover: cell continuity; cell diversification; characteristics of cell interfaces. Further information can be obtained from Montrose J. Moses, Acting Secretary, American Society for Cell Biology, Box 2982, Duke University School of Medicine, Durham, North Carolina.

THE Food and Agriculture Organization is sponsoring an international conference on "Fish in Nutrition" in Washington, D.C., during September 19–27. The programme will include: the role of fish in world nutrition; chemical composition of fish and fishery products; contribution of fish and fish products to national diets; fish and fishery products in animal nutrition; demand for fish as human food and possibilities for increased consumption. Further information can be obtained from the headquarters of the Food and Agriculture Organization, Viale delle Terme di Caracalla, Rome.

A COURSE for young naturalists with a strong interest in conservation is run annually by the Verein Naturpark in the Lüneburger Heide, North Germany. The participants are given first-hand knowledge of the theoretical and practical sides of conservation. Information can be obtained from the camp secretary of the International Youth Federation for the Study and Conservation of Nature, Reiner Lidauer, Paracelsusstrasse, 6, Salzburg, Austria, or from Derek S. Davis, 56 Altmore Avenue, East Ham, London, E.6, of the British Co-ordinating Group of the International Youth Federation.

ERRATUM. In the article entitled "Effect of Polyvinyl Alcohol on the Distribution of Iodine-131 in the Internal Organs, Fluids and Excreta of Rabbits", by H. F. Nowak and J. Kucharski, in *Nature* of August 12, p. 665, the sentence beginning at line 14 from the end should read: "Administration of iodine-131 with polyvinyl alcohol caused it to collect in smaller quantities in the parenchymal organs and thus the possibility of injury to these organs is considerably reduced".

INORGANIC POLYMERS

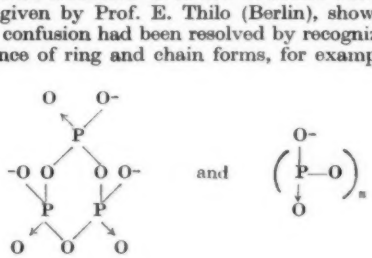
THE international symposium on "Inorganic Polymers", held at the University of Nottingham during July 18–21, attracted more than four hundred people, drawn from eighteen different countries. This response reflected the widespread interest in a subject virtually unmentioned a few years ago.

Contributions to the symposium were of two kinds: nine invited speakers gave full-length lectures, the texts of which are to be published by the Chemical Society; thirty-one shorter communications were presented and very briefly discussed. The most effective discussions were those which were held informally between sessions: these were facilitated by the fact that nearly all those attending were accommodated on the University campus. These social arrangements were excellent, and contributed in no small measure to the undoubted success of the meeting.

After the president of the Chemical Society (Sir Alexander Todd) had welcomed visitors in English, French, German and Russian, an introductory address was delivered by Dr. J. S. Anderson (National Chemical Laboratory). A main theme of this address was the problem, very fundamental to the symposium: "What is an Inorganic Polymer?" The theme recurred from time to time, and led to good-humoured exchanges suggesting rivalry between organic and inorganic chemists over polymers which might be described as organo-metallic, metallo-organic, or inorganic. Anderson pointed out that there are really two parts to this problem: (*a*) How large must the molecule be to be described as a polymer? (*b*) What restrictions of chemical composition are implied by the adjective 'inorganic'? On the first point, cyclic structures such as $(PNCl_2)_3$ occupied a prominent place in the discussions but are neither more nor less polymeric than, say, benzene. The second point is best dealt with by listing some of the types of polymer which were discussed during the meeting. They fall naturally into three classes: (1) polymers containing no carbon, for example, the chain forms of sulphur and selenium, $(PNCl_2)_n$, polyphosphates; (2) chain polymers in which the main chain does not involve carbon, for example, the siloxanes $(SiR_2—O—)_n$, where R is organic; (3) chain polymers in which carbon forms only a part of the main chain, for example, co-ordination polymers of the type $(—M—R—)_n$, where M is a metal and R an organic unit. The best compromise definition might insist on the absence of carbon from the main chain, and then include (2) but not (3). In point of fact, the wide range of materials surveyed by different speakers reflects the origin of much of the recent work in this field in a search for polymers capable of practical use at high temperatures. Since the object is to improve on the performance of organic polymers, large organic groups have been automatically excluded from consideration.

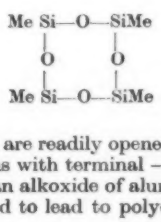
Before reporting on papers which dealt with specific polymers, one other general lecture may be mentioned. Prof. G. Gee (Manchester) directed attention to the large number of the systems under discussion which could exist in either chain or cyclic forms, the latter containing usually 6 or 8 atoms in the ring. He outlined a thermodynamic analysis of the equilibrium between ring and chain forms, pointing out that both heat and entropy of conversion ring/chain were likely to be small. In consequence, the equilibrium is readily displaced in either direction by small influences, and four classes of behaviour are possible: (i) chains unstable at any temperature; (ii), (iii) chains unstable below a 'floor temperature' or above a 'ceiling temperature'; (iv) an equilibrium chain concentration on any temperature. This analysis cannot yet be widely applied, due to lack of thermochemical data. In most of the systems described, equilibrium is not readily attained, and the product found in given circumstances is determined by kinetic factors.

One of the classical problems of inorganic chemistry is that of the structure of the many phosphates which have been described. A review of recent work in this field, given by Prof. E. Thilo (Berlin), showed that much confusion had been resolved by recognizing the existence of ring and chain forms, for example:



where n could reach values as high as 10^6. The chain forms may be either amorphous and soluble in water or crystalline and insoluble. The nature of the cation is of importance, in controlling the configuration of the chain and the crystal structure. The arsenates form a similar family, and mixed compounds, both cyclic and chain, can also be made.

Among the very few synthetic inorganic polymers which have already achieved industrial success the siloxanes occupy a prominent place. There have also been many attempts to modify the siloxanes by partial or complete replacement of silicon by other atoms. The lecture given by Prof. K. A. Andrianov (Moscow), who has been outstanding in this work, summarized some of his methods and results. Cyclic siloxanes such as $(SiMe_2O)_4$ are very well known, and Andrianov has described three-dimensional closed structures, including one which may be written as a cube having as its side:



Cyclic structures are readily opened by strong bases to give linear chains with terminal —O⁻. Reaction of such a chain with an alkoxide of aluminium, titanium or boron is reported to lead to polycondensation, for example:

$$HO—(SiR_2O)_nH + Al(OR')_3 \rightarrow HO—[(SiR_2O)_n—Al(OR')O]_xR$$

Vulcanizable elastomers having good physical properties have been reported.

A contributed paper by D. L. Alletson, A. G. Davies and B. N. Figgis (University College, London) described work on the dialkyl tin oxides, indicating the existence of tin analogues of both the ring and chain siloxanes. Four-membered rings are believed to occur among the stannoxanes.

Another well-known group of inorganic polymers are the phosphonitrilic compounds, of which the most familiar is the chloride $(PNCl_2)_n$, sometimes referred to as 'inorganic rubber'. In his lecture, Dr. C. P. Haber (U.S. Naval Ordnance Laboratory, California) summarized recent work, including several new general methods of synthesis. The usual procedure for the chloride involves reaction of ammonium chloride with phosphorus pentachloride (PCl_5), and can be modified to give $(PNR_2)_n$ by replacing PCl_5 by PCl_2R_3. This method always gives cyclic products; in the case of the chloride these are readily polymerized by heating. The chain polymer $(PNR_2)_n$ (R=Me, Et, Ph) cannot, however, be obtained in this way, as the ring does not open on heating. Reaction of $PClR_2$ with sodium azide leads, via R_2PN_3, to chain polymer. Substitution reactions can also be used to replace chlorine by other groups. By these various techniques, a considerable range of both cyclic and polymeric phosphonitrilic compounds have now been made.

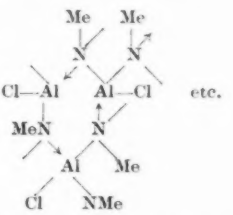
One of the most interesting problems associated with the phosphonitrilic compounds concerns their electronic constitution, that is, the nature of the P—N bond. In a contributed paper, N. L. Paddock (Manchester) reviewed this problem in the light of recent thermochemical and spectroscopic measurements and studies of base strength. There is no doubt that delocalized bonds are formed, both in rings and in the chain, by overlap of p orbitals of N with d orbitals of P. It is possible, on this basis, to understand why the stability of a PN ring is very much less dependent on its size than is that of a hydrocarbon $(CH)_n$. This problem was also referred to by Prof. A. B. Burg (Southern California) in his lecture. Discussing the polymerizability of the 6-membered ring $(PX_2N)_3$, Burg sought for factors tending to increase the size of the angle PNP, and on this basis deduced as the order of increasing polymerizability: $X = NR_2 <$ Me or Ph $<$ Cl $<$ F or CF_3.

Turning to BN polymers, Burg reported that the chain polymer $(Me_2N.BH_2)_n$ is unstable at room temperature, but the 6-membered ring ($n = 3$) is unchanged by heating at 300° C. The structure of this compound has been determined (by Lipscomb) and found to be of cyclohexane chair form, with ring angles of 113–114°. The corresponding phosphino–borine $(M_2PBH_2)_3$ is similar, with ring angles 118° (P) and 112° (B).

BN polymers were further discussed in the lecture by Prof. A. W. Laubengayer (Cornell) who represented three stages arising by progressive removal of RX from a co-ordination compound I of R_3N and BX_3:

$$\underset{\text{I}}{R_3N \rightarrow BX_3} \rightarrow \underset{\text{II}}{(R_2N \rightarrow BX_2)_n} \rightarrow \underset{\text{III}}{(RN—BX)_n} \rightarrow \underset{\text{IV}}{(BN)_n}$$

In series II, both B and N are approximately tetrahedral, but there is charge alternation in the ring or chain. Series III has as its parent member borazole (NH—BH)$_3$, which possesses a plane aromatic ring and closely resembles benzene. The corresponding chain is unknown: on pyrolysis borazole gives a variety of products, among which have been identified analogues of naphthalene and diphenyl. Boron nitride (IV) forms a layer structure very similar to graphite, but with B^- and N^+ alternating in adjacent layers. Aluminium–nitrogen polymers have been less studied, and there is no evidence for a borazole analogue. (Me N. AlCl)$_n$ is a very stable, insoluble material, possibly:

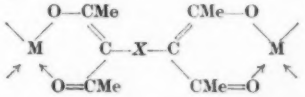


Several contributed papers also dealt with BN polymers. W. Gerrard and E. F. Mooney (Northern Polytechnic, London) reported that borazole ring formation could be prevented by bulky substituents (III, R = P or Bu, X = Ph). J. Dewing (Imperial Chemical Industries, Billingham) reported the polymerization of $Me_2N.BH_2$ by heating at 150° C. and 3,000 atm., to an amorphous, infusible, insoluble polymer, which slowly reverted to a cyclic dimer under ambient conditions.

The two remaining lectures, by Prof. J. C. Bailar (Illinois) and Prof. O. Schmitz-Dumont (Bonn), both dealt with co-ordination polymers. The underlying principles are straightforward, though their application to produce useful polymers has not yet been successful. The most useful technique involves polymerization by co-ordination, and requires the use of a bi- or poly-functional ligand. A very simple example is

$$Ag^+ + NH_2(CH_2)NH_2 \rightarrow$$
$$\rightarrow Ag^+ \leftarrow NH_2(CH_2)_2NH_2 \rightarrow Ag^+ \leftarrow$$

A metal of higher co-ordination number can form chelate rings; use of *bis*-diketones then leads to very stable polymers such as



where X is any linking unit such as $(CH_2)_n$ or C_6H_4. A variation on this procedure involves linking together, for example, by polycondensation, units which are already co-ordinated, that is, making the X bridges. It will be seen that the 'inorganic' content of such a structure is extremely small.

In this review of the symposium, no attempt has been made to mention all the contributors or all the topics discussed. It will be clear that the emphasis was on the preparation of polymers. The symposium offered a timely opportunity to take stock of the present position, and should lead to some reorientation of future effort. While there is clearly much synthetic work still to be done, increasing attention should now be given to reaction mechanisms and energetics, and to the physical study of the products. Prof. C. C. Addison and his colleagues on the organizing committee are to be congratulated on a most successful venture, which may well exercise an appreciable influence on the development of the subject.

G. Gee

SOCIETY OF EUROPEAN NEMATOLOGISTS

At the invitation and under the presidency of Prof. J. van den Brande, the sixth international nematology symposium of the Society of European Nematologists was held during July 24–28, 1961, at the State Agricultural College in the ancient and beautiful city of Ghent. From some twenty-seven countries came about 140 participants, the largest contingents being those from Belgium (thirty-three), Great Britain (thirty, after deducting four for overseas students), Germany, West and East (nineteen), and Netherlands (seventeen).

After the inaugural presidential address and a stimulating special lecture by Prof. de Coninck on "Problems of Systematics and Taxonomy in Nematology today," there followed some fifty papers, grouped after the first day under two simultaneous sections, and each ostensibly limited to 20 min., including time for discussion.

Lack of space precludes a reference to every paper, but some of the trends and highlights can be briefly indicated.

In the field of taxonomy a paper by B. G. Chitwood (presented in his absence by A. L. Taylor) described and illustrated photographically the holotype and allotype specimens of most of the recognized *Meloidogyne* species and sub-species. These specimens were selected in 1960 by a committee of the American Phytopathological Society. Chitwood regards these taxa as possibly polyphyletic, originating as repetitive mutants; at least one (*M. javanica javanica*) is probably a defective triploid.

W. C. Clark commented on the variability and accuracy of linear measurements used in systematics, and of ratios based on them. The de Man ratios, based on total length, are of little value when the latter is highly variable; also, they presuppose absence of allometric growth. H. Gysels showed that in *Panagrellus silusiae* post-embryonic growth is in fact allometric with respect to length: moreover, in the expression $y = Kx^a$, the index may change at different stages in the life cycle.

Among matters of life-history and biology, A. M. Shepherd showed that *Heterodera göttingiana* larvæ emerged from cysts in soil, in the presence of a host, even more readily than *H. rostochiensis*, but could not be made to emerge *in vitro*. In tissue-culture experiments with *Meloidogyne*, M. L. Schuster and R. M. Sandstedt found that movements of larvæ were consistent with a random walk and accidental contact with roots rather than with an attraction mechanism. W. H. Lavallee and R. A. Rhode, on the other hand, interpreted the rapid concentration of *Pratylenchus penetrans* around a host at one end of a box as due to attraction.

The orientation of *Ditylenchus dipsaci* to physical stimuli was discussed by H. R. Wallace: in gradients of temperature, moisture, and size of sand particles, the worms preferred 10° C., high moisture, and fine sand.

The feeding mechanisms of Rhabditids and Tylenchids were interestingly compared in a film by C. C. Doncaster. The movements of dye globules and air bubbles through the œsophagus were analysed, the bubbles showing change in size with pressure. He also showed a film on nematode predators.

The geographical or topographical distribution of nematode populations was discussed in several papers. M. Ritter was able to correlate the distribution of species of *Meloidogyne* in France with climatic zones. J. de Maeseneer reported on the distribution of some of the chief migratory root nematodes in Belgium. E. Gadea gave data on the free-living nematodes of freshwater and moss biotopes in Spain. B. Weischer has made the ecologically interesting discovery of two species of Desmoscolecidae in vineyard soils from a small area centred on Bad Ems: the point is that most other species of this family are marine. A. B. Oteifa usefully summarized the chief plant nematodes of crops in Egypt.

Changes in nematode populations were dealt with by S. Stemerding, who counted *Tylenchorhynchus dubius* and *Rotylenchus robustus* in weekly samples from a monoculture of peas. R. Gair *et al.* found differences between varieties of spring barley in their effect on rate of increase of *Heterodera avenae*. M. Oostenbrink presented the results of examining numerous samples from meadows and arable land in the Netherlands. It is clear that the heaviest populations of nematodes come from the grass/clover crop from meadows. Many of these nematodes are plant parasites and are important in two ways: some are harmful to the grass/clover crop itself, and some become harmful on arable crops when the meadow is ploughed. K. Kuiper is tracing the gradual invasion of new polder-soils from the Zuider Zee area by plant nematodes, and the factors responsible for this invasion.

In the field of pathology, H. Goffart has shown that one effect of *Ditylenchus dipsaci* in sugar beet is to increase the content of reducing sugars; this also occurs with *D. destructor* in nutrient-agar cultures and to a smaller degree with *Heterodera rostochiensis*. The effect is ascribed to enzyme action from the œsophageal glands.

The importance of nematodes as vectors of virus pathogens was stressed by R. S. Pitcher. All the known cases belong to the Dorylaimoidea and are of two types: some 'ring-spot' viruses carried by nematodes of the Longidorinae, and tobacco rattle virus carried by *Trichodorus*. H. H. Sol showed that *T. pachydermis* appears to be the only transmitter, nematodes extracted from infected soil retaining the virus for at least three weeks.

An interaction between *Verticillium albo-atrum* and *Pratylenchus penetrans* has been shown by W. B. Mountain and C. D. McKeen, the nematode being more prevalent in the presence of the fungus.

L. Roguska-Wasilewska found that *Heterodera schachtii* inhibited the respiration of rape far more than did the saprophagous *Eucephalobus elongatus*.

Other disease situations included: *Ditylenchus dipsaci* increasingly causing collar-rot of beet in Switzerland (A. Savary); *D. radicicola* of Dutch strain damaging *Poa annua* but not barley, whereas the Swedish strain behaves in the reverse manner (J. J. s'Jacob); *Radopholus similis* causing root-rot in banana in New South Wales (C. D. Blake).

Numerous papers dealt with control methods. Outstanding were a tabulated compilation of reported crop-yield increases as a result of using nema-

ticides in the United States, comprising 853 comparisons (A. L. Taylor), and a succinct account of the way in which *Heterodera rostochiensis* is held in check on Long Island by nematicidal treatment (chiefly with dichloropropene), cultural methods and legal regulation, and the use of resistant varieties of potato in fields where the nematode population remains below the level of discovery (W. F. Mai and M. B. Harrison).

The diffusion of ethylene dibromide through soil was shown by F. Call and N. G. M. Hague to be largely determined by soil porosity; relating chemical to bioassay determinations, they found that very low concentrations persist for long periods. H. W. Nolte found only temporary benefit from using 'Vapam' against *Pratylenchus* in pine nurseries, and K. Raschke showed that reduction of hatching from *Heterodera* cysts after treatment with 'Trapex' was due not to delayed hatching but to death of larvæ.

Ultrasonic vibrations, tested by L. Kaempfe, gave disappointing results, especially against encysted *Heterodera* larvæ.

Radiotracer techniques using phosphorus-32 were applied by B. A. Oteifa and D. M. Elgindi to a study of galling in tomatoes by *Meloidogyne javanica* and its effect on uptake of nutrient, and by F. Sprau and A. Suesz to indicate that *Longidorus maximus* feeds on the roots of beet and is thus a parasite; and, using carbon-14, were adapted by B. M. Zuckerman to estimate the amount of plant fluid ingested by a single nematode.

At the biennial general meeting on July 27 members of the Society heard an encouraging report from the secretary, Dr. Lindhardt, who was able to show a steady growth in membership over the years:

1957: 141 members from 19 countries; 1959: 179 from 25 countries; 1961: 218 from 33 countries (of this last total, 166 come from Europe).

An invitation from Dr. John Grainger to hold the seventh symposium at Auchincruive in September 1963 was accepted with enthusiasm, and Prof. Nolte tabled an advance invitation to Aschersleben for 1965. It was decided to issue to members a duplicated 'Newsletter' under the editorship of Dr. Lindhardt, assisted by a corps of reporters.

A lavish social programme included a civic reception at the Town Hall, a dinner at the Castle of Laarne, and a lunch at the Park Hotel, Lokeren. There was also a programme of daily excursions for non-participating ladies.

Translator-in-chief, displaying equal facility in the three official languages besides his native Flemish, was Mr. R. Kips (secretary).

B. G. PETERS

A NEW LABORATORY FOR RESEARCH ON CREEP

ON July 6 the new Creep Laboratory of the British Electrical and Allied Industries Research Association was opened at Leatherhead by Dr. Charles Sykes, managing director of Thomas Firth and John Brown, Ltd. Its purpose is to carry out an extended programme of long-term co-operative research and investigation on the creep of steels in conditions of high temperature and mechanical stress.

Increase in the overall efficiency of generation of electrical power by steam now turns critically on improving the thermodynamic efficiency by operating at higher temperatures and pressures. The limiting factors in practice are set by materials capable of withstanding these conditions during long periods. The highest temperatures (above 600° C.) are found in superheater tubes, steampipes and control valves, but conditions are even more onerous in turbine rotors, blades and casings where tolerance to distortion is very small.

Historically, progress in steel development has occurred in three phases. Initially, power-station plant was constructed entirely of plain carbon steel; this was followed by the addition of small amounts of alloying elements which added strength to the steel. Now, in the third phase, special highly alloyed stainless steels are being developed. The cost of such steels is high and is reflected in the great increase in the proportion of cost of steam-raising equipment in modern power stations. Such increases must, in the long run, be offset by increased efficiency, and this can only be judged when full knowledge of the life and reliability of these new steels is available. Creep, which can be defined as permanent deformation of materials arising from continued stress, is of basic importance in this connexion.

The investigation of the properties of high-temperature steels for power stations was inaugurated as long ago as 1930 by the Steels for High Temperature Committee of the Electrical Research Association. Most of the investigations until recently have been carried out by the Metallurgical Division of the National Physical Laboratory and at the National Engineering Laboratory. In 1957 work was started at the Electrical Research Association to supplement that at the National Physical Laboratory. The National Physical Laboratory is, however, no longer able to cope with the vastly expanded programme, and through the joint efforts of the Committee it has been possible to centre all the work in the new buildings and equipment installed at Leatherhead.

The main Laboratory is 140 ft. long and 45 ft. wide. It has space for about 250 units for testing creep. Since these units dissipate considerable heat it has been necessary to install air-circulating equipment to maintain a suitable ambient temperature for instrumentation and personnel. The bulk of the units are designed to record either the creep or the time of rupture of standard samples cut and very accurately machined from bars of steel or from fabricated parts. At present there are fifty units of high sensitivity fitted with Martens optical extensometers. These machines are designed particularly for studying the creep behaviour of turbine rotor forgings, blades and casings, and are at present also being used for the creep of metal weld deposits and the parent metal. There are fifty large rupture machines, in which ten specimens can be stressed simultaneously end to end. They do not record the creep but only the time to rupture. They are used to provide long-time design data for high-alloy steel steampipes and superheater tubes. The stress applied to the specimens is varied so as to produce rupture times extending from about 300 to 10^5 hr. Graphs of rupturing time against stress are then used

to predict the life under the stress conditions found in service. The variation from cast to cast within a particular specification is under investigation.

There are a further fifty dual-purpose creep/rupture units which can be used either as specimen rupture machines or as creep machines using dial gauge extensometers where less-accurate creep measurement is required. They are at present employed in measurements on turbine casings. One large machine with 15-ton loading has been installed for rupture-testing of full sections of steampipe.

The temperatures of the specimens are maintained by electric furnaces, the range of temperature of which at present extends from 450° C. to 700° C., but can be raised above this temperature when future requirements demand. The furnace temperature is maintained automatically by an electronic controller, to within ± 3° C. up to 600° C. and to within ± 4° C. above 600° C. Temperatures of the specimens themselves are measured by means of thermocouples (of which there are three on each specimen) in the high-sensitivity units. The thermocouple readings can be channelled selectively to a central control desk for manual measurement, but during critical periods they can be connected to one of 36 continuous recording charts situated in the Laboratory. In the rupture units there are five thermocouples distributed among the ten specimens. These, too, can be channelled to the control desk.

Another important project which is being undertaken in the new Laboratory is the testing to destruction of thick-walled tubes by applying steam pressure for long periods of time at temperatures encountered in service. This enables the creep data obtained in the Laboratory to be correlated with data on actual tubes so that the expected life in practice can be assessed. In testing creep in the Laboratory only a single uni-axial stress is applied whereas a steampipe is subjected to a complex stress system. Four such test rigs have been operating in the Electrical Research Association during the past three years. There are now fifteen rigs.

The data obtained in the new Laboratory will be subject to constant scrutiny as a means of providing more understanding of the mechanism of creep resistance of high-temperature steels. Knowledge of this kind will almost certainly be of benefit in the future development of steels for still higher temperatures, the demand for which is likely to continue for a long time.

CLIMATOLOGY AND CONSERVATION IN MUSEUMS

THE Commission for Museum Laboratories of the International Council of Museums and the International Study Centre for the Preservation and Restoration of Cultural Property have recently undertaken a world-wide inquiry into the subject of environment in relation to conservation. Replies from sixty-four institutions to a questionnaire on the subject, and further study of the situation thus revealed, form the basis of a 46-page report *. In it, museum curators, rather than scientists specializing in conservation, are provided with a useful compendium on the atmosphere and its contaminants, hygrometry, instrumentation and equipment for the control of museum climate. In addition there are several useful reference tables relating to conditions within and outside museums in various parts of the world.

As is proper for documents issued by an international organization, several authors from various European countries have contributed, but the actual publication consists of complete texts in French and in English. Taking into account the polyglot sources of the material, the effort can only be said to have been altogether praiseworthy. Moreover, the fact that the report is written for curators who must be assumed to have little knowledge of the physics involved, must have added to the task. Faced with this requirement, however, it is unfortunately all too easy to stray away from rigorous scientific exposition for the sake of a tempting analogy. For example, it may be acceptable in the introductory paragraph to compare inanimate objects with human beings in their reaction to the environment: "They live and breathe in the sense that there is continual if imperceptible change arising from their *adaptation* to the ambient conditions of the museum"; but, is it wise to persist with: "Some objects have a surprising capacity for *adapting* themselves to environment apparently without deleterious effect. Thus, a particular piece of wood or bronze may be recovered in remarkable condition after having been conserved for centuries in a swamp or bog; or a certain picture which has been exposed in a damp church may survive the ill-effects beyond all expectation. Let them be brought from their accustomed *habitat* into a museum, however, and the true state of affairs will soon be manifest, even where the atmosphere is rigorously conditioned" ? (p. 243). Again: "The crux of the matter is to be found in the past history of the specimen, its structure and the conditions to which it has already become *acclimatised*. As is well known, even the best museum conditions can be dangerous for a painted panel that has been normally housed in a cold, damp country house" (p. 253). This may or may not be a phenomenon which is observable under scientific control, but if it is, to imply a conditioning process in the biological sense only helps to perpetuate the notion which is sometimes present among curators that objects, particularly paintings, lose their 'resistance' in the soft environment of the air-conditioned gallery and decay, or alternatively, become unfit to withstand uncontrolled conditions.

Where the equilibrium that the object will attain in the museum results in a considerable change in moisture content, there will obviously be undesirable —often catastrophic—volume changes. Special techniques have been developed for dealing with such cases as, for example, water-logged wood. But it is difficult at first to entertain the idea that stabilizing the atmosphere at parameters well within those of the natural cycles can be harmful. Evidence on the alleged phenomenon is unfortunately biased for the simple reason that objects are closely and regularly inspected once they have entered the museum—but

* International Centre for the Study of the Preservation and the Restoration of Cultural Property, Rome. Works and Publications III: *Climatology and Conservation in Museums*. (Reprinted from *Museum*, 13, No. 4, 1960.) Pp. 201–289. (Rome: International Centre for the Study of the Preservation and the Restoration of Cultural Property, Via Cavour, 256, 1960. Also available at H.M.S.O.)

not before. Even so it is not without interest to proceed on hypothetical lines because it is the experience of every curator that certain objects prove intractable. It might be supposed, for example, that continual swelling and shrinking prevents embrittlement in organic materials. Another possibility is connected, not unreasonably, with gradients in moisture content within the object. Certain forms of deterioration in paintings (both panel and canvas) can be shown undoubtedly to result from the gradient set up when the back is in close proximity to what may be a cool and/or damp wall, while the front enjoys constant and ideal but different conditions. It cannot be assumed that such steady-state conditions are *a priori* more favourable on all counts than cyclic ones in which even a reversal of the gradient is possible. The transfer of soluble material, whether salts or adhesives, within the object is a source of breakdown, particularly at an interface where the structure is laminar. The report does not deal with this phenomenon beyond pointing out that the variation in climate within a room may be such as to produce a 12 per cent variation in relative humidity over 3 m. in height. The importance of moisture content gradients relative to the main hazard—the swelling and shrinking of hydrophilic gels—is a matter for investigation. The result may well explain the unexpected results of air-conditioning which sometimes disappoint the curator. In so many investigations connected with conservation the actual number of variables is difficult to define, and generalizations are often misleading. A case in point is the assumption (p. 247) that picture canvases become slack when the atmosphere is over-dry, as raw natural fibre textiles do. The converse is more often observed because the textile behaviour is opposed by that of the animal glue with which it is impregnated—old masters made use of this effect to tension their canvases.

The report is generously illustrated with diagrams, photographs of instruments and conditioning apparatus, and with striking examples of objects which have suffered deterioration due to unfavourable environment.

S. Rees Jones

VIEWING AND LISTENING

A YEAR ago the British Broadcasting Corporation published a report of an audience research inquiry under the title *The Public and the Programmes*. The interest which this aroused has encouraged the Corporation to make available other findings of its Audience Research Department. *Facts and Figures about Viewing and Listening** offers, in a series of twelve charts, some facts which are important to a proper understanding of what has been happening to viewing and listening habits in recent years.

Each chart has its accompanying commentary explaining the significance of the matter dealt with and directing attention to outstanding points.

Charts I and II show the extent to which the British public uses the three broadcasting services, BBC Sound, BBC-TV and ITV. This is assessed in two ways—in terms of the numbers who typically listen (or view) in the course of a single day (that is, the numbers of 'patrons'), and in terms of the average number who are listening (or viewing) at any given moment (that is, the 'average audience').

Chart I, "The Patronage of Broadcasting", shows the number of adult 'patrons' for each service quarter by quarter for the past three years and shows that the number of adult patrons of BBC Sound has not fluctuated very greatly (this, despite the continuing increase in television coverage). The numbers of adult patrons of both BBC-TV and ITV have shown a rising tendency, with a seasonal fluctuation. The number of BBC-TV adult patrons is consistently above that of ITV because, if for no other reason, though all viewers can see BBC-TV, some cannot yet receive ITV.

The curves for child-patrons show that a much smaller proportion of children than adults patronize Sound and a larger proportion of children patronize ITV than patronize BBC-TV.

In Chart II a different picture emerges when the services are compared in terms of 'average audience' than when they are compared in terms of 'patrons'. Thus, Sound's patrons exceed those of either television service, but its average audience is much smaller than that of BBC-TV or ITV. The explanation lies in the fact that the average patron of sound listens for a relatively small proportion of the time during which sound broadcasting is transmitted. Similarly, though there are more patrons of BBC-TV than of ITV, the former spend less time viewing (1 hr. 44 min. a day) than do the latter (2 hr. 12 min.); hence the number who are viewing ITV at any given moment (7,500,000) is greater than the number who are viewing BBC-TV (6,350,000).

Chart III shows the relationship, since 1952–53, between the amount of time (in the evening) during which television programmes were broadcast and the amount of time which the average viewer actually spent in viewing. Two points emerge from this comparison: (1) that an increase in the number of television programme hours in the evening is not followed by a proportionate increase in viewing; (2) that, since commercial television became available, there has been little difference between the amount of time spent in evening viewing by those who could receive both BBC and ITV and by those who could only receive BBC-TV.

These results suggest that the introduction of further television services (a third and perhaps a fourth) will be unlikely to cause an increase in the total amount of time which people devote to viewing.

Charts IV and V illustrate the way in which people with multichannel receivers distribute their viewing between BBC-TV and commercial television. Chart IV shows the proportions which, on the average day in each quarter of the past two years, confined their viewing to BBC-TV, viewed both BBC and ITV, confined the viewing to ITV, or did not view at all. The last of these categories tends to vary seasonally, being greatest in the July–September quarter. Apart from this, there has been a tendency in the past two years for fewer adults to confine their viewing to ITV and for considerably more to view both services. Among children, many more restrict their viewing to ITV than restrict it to BBC-TV.

*British Broadcasting Corporation. *Facts and Figures about Viewing and Listening.* In Twelve Charts with a Commentary. Pp. 31. (London: British Broadcasting Corporation.) 5*s.* net.

Chart V shows how Band 1/3 viewers have divided their evening viewing time between BBC and commercial television. The trends are remarkably similar: when commercial television first comes to a region there is usually a period of 'settling down' during which the proportion of time given to BBC-TV tends to fall. Then relative stability is reached.

Chart VI relates to the way evening viewing is divided between BBC and ITV according to the educational level of the viewer. Among those whose full-time education continued up to and beyond the age of seventeen years, roughly half view BBC-TV either virtually exclusively or more than they view Independent Television. Those in this group who view ITV exclusively or predominantly are a minority, 37 per cent. In the group whose full-time education was not continued beyond the age of fifteen or sixteen, about 30 per cent either always view BBC or view it more than ITV, whereas 52 per cent, always or more often, view ITV. In the group whose full-time education ceased at fourteen years or earlier the corresponding proportions are 20 per cent and 65 per cent respectively.

Chart VII shows the present comparative quantities of listening to sound broadcasting at different times of the day. The period when there is most listening is shown to be that between noon and 2 p.m. when the average audience is nearly 5·5 million. The main point brought out by the chart is that 'levels' of listening in the afternoon and evening are now far lower than those in the morning; in the evening sound broadcasting must compete with television.

The 1950–60 trends in the day-time and evening audiences for sound are shown in Chart VIII. During the period 6–11 p.m. Sound's audiences declined sharply each year with the result that, for every four people who were listening in January–March 1950, only one was doing so ten years later. Between 9 a.m. and 6 p.m., on the other hand, when the competition of television was less fierce, audiences for sound showed no comparable decrease. For every four people listening in the winter of 1950, three were doing so ten years later.

Chart X shows how much time 'listeners' and 'viewers' have spent with sound broadcasting in the evening each autumn since 1954. The average adult 'listener', far from reducing his evening listening, has tended to increase it in the past six years. He now listens for about 1 hr. (out of a possible 5) a night. The average 'viewer', on the other hand, has never listened much in the evening. This small average quantity of listeners guarantees Sound broadcasting a considerable audience in the evening. Even if the time should come when virtually everyone has a television set, the audience for sound broadcasting in the evening would still, at this rate of 'viewer-listening', be about one million persons.

Chart XI shows the extent to which, during October–December 1960, children aged five to seven, eight to eleven, and twelve to fourteen years viewed television between 5 and 6 p.m. and in the first half of the evening, 6–9 p.m. Each day between the hours of 5 and 9 p.m. the average child of five to seven years and the average child of eight to eleven years, watched television for more than two hours, while the average twelve to fourteen-year-old watched for nearly an hour and a half—and in each case the bulk of this time was spent in viewing programmes after 6 p.m.

Between 5 and 6 p.m., the older the child the greater is the share of such time when he views which he devotes to BBC-TV. During the early evening, on the other hand, the reverse is true: the older the child the smaller is the share of his viewing time which he spends with BBC-TV.

Chart XII shows that the size of an audience is, by itself, no sure guide to its popularity. The Chart illustrates this by showing that, during four typical weeks in mid-winter 1960, there were fourteen BBC-TV broadcasts each of which had an audience numbering approximately six million persons yet varied widely in the extent to which they were enjoyed. At one extreme one broadcast had a reaction index (the BBC's yardstick of 'enjoyment') of seventy-nine (out of a possible one hundred), while at the other was one with a reaction index of forty-nine. Thus, had 'audience size' alone been accepted as the criterion of popularity, these two broadcasts would have ranked as equal, whereas it is clear that those who saw Broadcast No. 1 enjoyed it far more than those who saw Broadcast No. 14.

EDUCATION OF BACKWARD CHILDREN IN BRITAIN

SINCE the War there has been a three-fold increase in the number of places available in special schools in Britain for educationally sub-normal children, the figure having risen from 11,000 in 1946, to 34,560 at the beginning of this year.

Educationally sub-normal pupils are defined as "pupils who, by reason of limited ability or other conditions resulting in educational retardation, require some specialized form of education wholly or partly in substitution for the education normally given in ordinary schools". They form one of ten categories of handicapped pupils for whom special education is provided. There is no overall national deficiency of places for blind, partially sighted, deaf, partially deaf, epileptic, physically handicapped, delicate and speech-defective children, although for some of these handicaps local improvements can be and are being made.

In a circular to local education authorities (11/61) Sir David Eccles, Minister of Education, estimates that a further 19,500 places will be needed to cater fully for all the educationally sub-normal children requiring education in special schools.

The Minister has already announced building programmes for the years up to March 1963 to provide about 10,000 of the places and intends to approve programmes, as soon as practicable, to provide the remainder.

The circular discusses the role of parents, teachers and doctors in discovering backwardness among children and in assessing their needs and the various measures that may be taken to enable them to live useful and independent lives. There has been a substantial increase in the rate at which children are brought forward for assessment since the War, during which time the school population has risen from 5 million in 1946 to 7 million in 1961.

Backwardness may be due to limited ability or other causes such as illness, a break in schooling or a disturbed home background. Many of these children

will not need the specialized treatment given in special schools and can be given suitable education in ordinary schools. On the other hand, boarding schools or boarding homes are required for a small minority whose special education needs to extend to their life outside the classroom, for children whose home environment is such that they are unlikely to make good progress unless removed from it, and for those who do not live within reach of a special day-school. Each child's needs should be carefully assessed and should be kept under regular review.

The provision of extra places in special schools and the increasing interest in the needs of backward children in ordinary schools will require a substantial increase in the number of teachers for the educationally sub-normal. Teachers who wish to specialize in this work can attend one-year courses in the education of handicapped children, which are mainly concerned with backward children. In 1962 there will be 16 of these courses taking 250 teachers in all; in addition, a number of shorter courses is available.

The adjustment from school to working life is more difficult for these children than for those of normal ability but can be made easier by the advice of the youth employment officers in finding suitable jobs and by sympathetic guidance and help whether in the form of further education or informal activities arranged by their former schools.

THE BUILDING RESEARCH STATION

THE open days, July 10–13, held by the Building Research Station, Garston, Watford, Hertfordshire, were primarily for the benefit of the building industry—architects, builders, surveyors and building materials manufacturers—leaving aside research in structural engineering, but much of the work displayed was, as might be expected, of general scientific interest. Indeed, the open days were coupled with the official opening by Sir Harry Melville, secretary of the Department of Scientific and Industrial Research, on July 7, of a new laboratory for physical and chemical research on building materials. The building has been erected by the Ministry of Works (superintending architect, Mr. H. A. Snow; superintending engineer, Mr. M. Woolfson; main contractor, Wilson, Lovatt and Sons, Ltd.) as part of the general building programme for the Station. It replaces more than a dozen war-time huts and brings together in one building all the laboratory research on building materials that the Station carries out. In opening the laboratory, Sir Harry Melville referred to the frequently expressed view that good research results most frequently come from unpromising surroundings and primitive apparatus. "Nothing", he said, "can be further from the truth in these days." He went on to point out that the scope of the work of the Station had broadened enormously since its foundation forty years ago, but that materials research is still basic to the whole of the building industry.

Although, as Sir Harry Melville said, materials research is the oldest part of building research, it has always been prosecuted with the best scientific tools of the day and with the most up-to-date techniques. Thus at present electron microscopy, X-ray analysis, infra-red absorption spectroscopy, differential thermal analysis, high-temperature microscopy and micro-waves all find application. The Station has indeed been a pioneer in the use of high-temperature microscopy directed to investigations of silicate minerals, and has developed a rapid and elegant technique for observing under the microscope reactions taking place at temperatures up to 2,400° C. A similar technique applied in the development of an X-ray camera for use up to 1,850° C. was also on view.

Micro-waves find application in equipment (now available commercially) designed to investigate the dampness of walls, a technique that is being further developed into a promising method of destroying dry rot by the heating effect of the waves, and radioactive techniques in the measurement of the wear of floor-surfacing materials.

Much of the research into building materials is devoted to a study of their fundamental physical and chemical properties, with the dual aim of providing a background of knowledge against which the durability of materials can be judged and of guiding new developments. Among the examples demonstrated were phase-equilibrium investigations on multi-component aqueous systems designed to increase understanding of the slow chemical reactions that materials undergo in use, and of the distribution of pore-size which has a close bearing on their resistance to frost. Laboratory abrasion tests for flooring materials have been notoriously unreliable, but following an investigation of the forces applied by the foot to the floor a new type of testing device was demonstrated that reproduces these forces.

New forms of construction make new demands on materials and throw up their own problems. Many curtain walling systems rely for their satisfactory performance on mastic materials for jointing. Thermal movements may be comparatively large and may make heavy demands on the mastic. Here the work of the Station has been mainly directed towards the assessment of the properties needed and the development of tests that can be used to evaluate the performance of jointing and caulking materials. The tests demonstrated dealt with rates of movement, stiffening with time, adhesion and load-bearing.

One of the great strengths of the Station is its ability to tackle most—if not all—aspects of problems involved in the development of new materials or processes. This is illustrated by its work on the perforated brick, the manufacture of which was demonstrated during the open days in the pilot-scale laboratory equipped with standard brick-making machinery. Not only has the Station been able to study the development of the design of the brick from the point of view of manufacture and æsthetic acceptability, but also it has been able to study strength, thermal properties, laying methods, rain resistance, and costs.

Visitors were able to see demonstrated the work of the Station on the packaging of building materials and the various units of mechanical plant developed for their handling; methods for fixing and joining plasterboard linings for house interiors, developed so as to remove as much of the wet process from building as possible; and the production of lightweight aggregate from pulverized fuel ash.

Of much current interest is the work on heating, as, for example, of flats, employing an electrical analogue, and of heat transmission through walls and roofs.

XUM

Equally topical is the survey being carried out of noise in London. One of the recording vans was on view, as was the main mobile acoustics laboratory, admirably equipped for almost any acoustical measurement and largely used for the measurement of sound transmission in buildings and for investigations into the laws governing the propagation of sound through the atmosphere.

The use of models finds considerable application in studies of the lighting of buildings, for, when suitably designed, they provide a ready means of obtaining both a factual and a subjective judgment of proposed schemes. The Station is active in establishing the principles on which permanent artificial lighting can be used to supplement day-lighting and thus give a new freedom to architectural planning. Closely associated with lighting is the use of colour in buildings: the one can modify considerably the effectiveness of the other. The range of colours for paint established as *B.S.* 2660 is now widely accepted, and the Station is engaged in trying to get the principles underlying the paint colours applied to building components such as tiles, linoleum, and plastics.

Allied to the physical investigations were the more subjective studies on the needs of users, illustrated by work on the design of drawing offices and of dwellings for old people.

Another use of three-dimensional models is found in the design of engineering services, and an exhibit showed ways in which they can help to resolve problems in the design of hospitals.

The economic aspects of building design and construction formed another exhibit on a topic of much importance. Here were shown investigations on the influence of the design of flats on their cost, the assessment of the comparative costs of different forms of urban development, and the programming of the sequence of work on building sites.

Altogether, there was much for the main target of the open days—architects and builders—to ponder over. The value of the open days for scientists is perhaps best exemplified by the director of a research organization in an entirely different industry, who went away having seen three pieces of work of immediate application in his own field. Some 3,500 visitors attended.

A NEW 'WEDGE' TYPE INSTABILITY OBSERVED IN A 'THETA' PINCH

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RECENT experiments by Niblett and Green[1] have shown that flutes are developed on the outer surface of a plasma produced by a theta pinch and these are interpreted as magnetohydrodynamic Rayleigh–Taylor instabilities produced by the acceleration of the interface separating the driving magnetic field and the plasma. Josephson has recently reported another experiment in which he investigated a possible mechanism for neutron production in a theta pinch with reversed trapped magnetic field[2]. Niblett has said (private communication) that in his experiments with Green they made detailed magnetic probe measurements and these showed that in their experiment parallel trapped field was obtained on the second half-cycle. In both experiments high-speed camera shots were taken of the end view of the plasma during the second half-cycle of the discharge.

We have been studying similar phenomena in theta pinches but have made our observations during the first half-cycle of the discharge. The theta piece used was 7·5 cm. long, 3·4 cm. internal diameter and this was wound around the middle of a quartz discharge tube 22 cm. long, 3·0 cm. diameter filled with deuterium gas at pressures of the order of 1 mm. of mercury. Our condenser bank had a capacity of 40 μF. and was charged to a voltage of 10 kV. An explosive type switch discharged the bank through the theta piece. The parameters of the experiment were such that we obtained[3] a cylindrically converging shock wave on the first half-cycle with trapped magnetic field parallel to the driving field.

Photographs of this discharge have shown an interesting new phenomenon; during the first half-cycle of the discharge we see bright radial 'wedges' with less luminous plasma filling their interstices. Even with radial wedges present it appears that there is still magnetic field trapped by the imploding plasma. About the time of maximum compression it forces its way out of the centre by pushing the plasma in the interstices out towards the wall of the discharge tube, thereby forming flutes which appear to have trapped magnetic field inside them. These outgoing flutes grow into a fascinating octopus pattern similar to that observed by other workers and associated with a rotation of the whole plasma. In our experiment the flutes grow in amplitude following a curved line giving an impression of rotation.

The number of wedges seems to be determined by the irregularities in the system since it can be varied by deliberately introducing irregularities in the external conductors. Figs. 1, 2 and 3 show high-speed framing camera records (about 8 frames per μsec.) of the theta pinch on the first half-cycle of the discharge. Figs. 1 and 2 are of theta pinches fired in the *Prize*[4] apparatus (Fig. 8), where it will be noticed that there are 16 wedges, that is, half the number of wires in the return conductor normally used for the *Z* pre-ionizer. Fig. 3 is taken with the return conductor removed and the theta piece wound directly on to the discharge tube, and shows that the wedges are randomly spaced and fewer in number; Fig. 3 is particularly interesting because it shows the trapped magnetic field apparently pushing its way out of the plasma through the three gaps where the plasma luminosity is least. The resulting outgoing flutes are also seen to grow as a result of the pressure exerted by the trapped magnetic field. A similar effect is also seen in Figs. 1 and 2. The field is observed to be trapped inside the largest flute until the fourth camera frame of Fig. 2, that is about $\frac{1}{4}$ μsec. after the first wedges become visible. In order for the diffusion time to be about $\frac{1}{4}$ μsec., the electron temperature must be of the order of 5×10^5 °K.

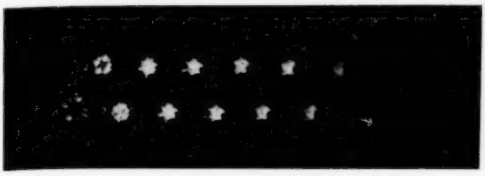

Fig. 1. Theta pinch with parallel magnetic fields. Ambient deuterium pressure 1 mm. mercury

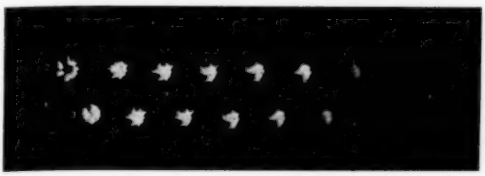

Fig. 2. Theta pinch with parallel magnetic fields. Ambient deuterium pressure 0·5 mm. mercury

Fig. 3. Theta pinch with parallel magnetic fields. Ambient deuterium pressure 0·56 mm. mercury

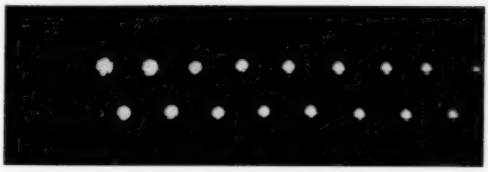

Fig. 4. *Prize* round ambient deuterium pressure 0·5 mm. mercury

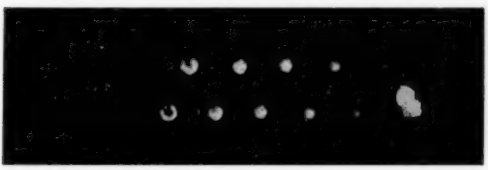

Fig. 5. Theta pinch with reversed trapped magnetic field. Ambient deuterium pressure 0·6 mm. mercury

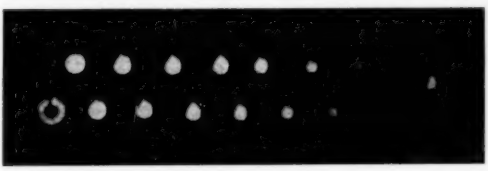

Fig. 6. Theta pinch with reversed trapped magnetic field. Ambient deuterium pressure 1 mm. mercury

The effect of reducing the trapped magnetic field by a considerable factor was studied by firing *Prize* with a 1 μF., 10 kV. condenser feeding the *z* pre-ionizer and a 40 μF., 10 kV. condenser bank feeding the theta pinch. Fig. 4 shows the result. It will be noticed that Rayleigh–Taylor type instabilities are formed during the implosion phase of the first half-cycle, but that they are damped out after the plasma converges on to the shock-tube axis; no outgoing flutes are observed after this time and better stability of the plasma is obtained.

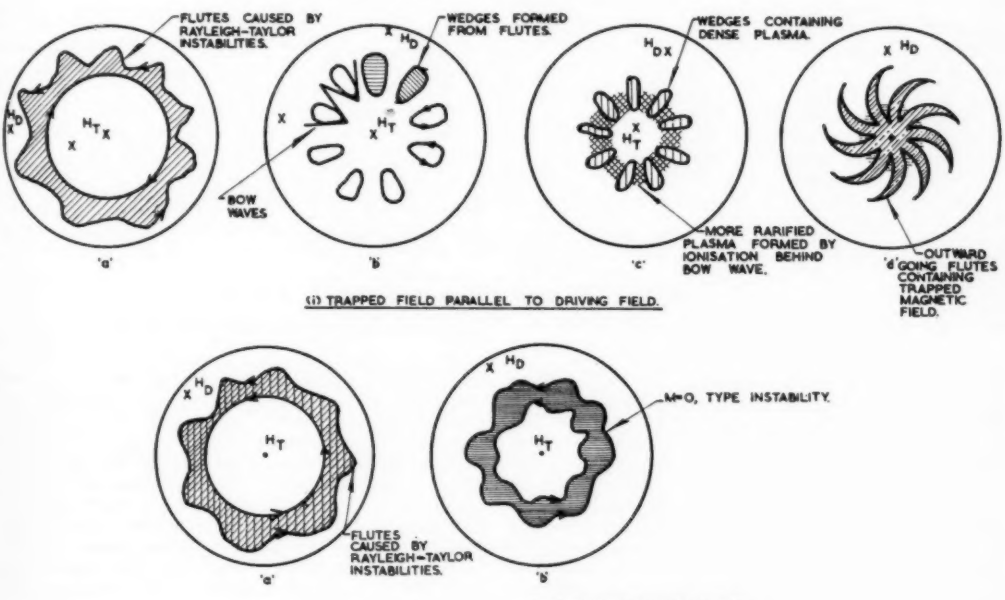

Fig. 7. Schematic diagram of instabilities observed in theta pinches

The next step was obviously to produce a reversed trapped magnetic field during the first half-cycle of the discharge. This was done with a pair of Helmholtz coils placed adjacent to the theta piece and fed from a critically damped circuit so that the rise time of the external field was 3 μsec. and the maximum field produced was 1,000 œrsted. When the externally produced field was at a maximum the theta discharge was initiated and the plasma produced had a reversed trapped magnetic field. Figs. 5 and 6 show the effect of a reversed trapped magnetic field on the discharge; it will be observed that there are no radial wedges or Rayleigh–Taylor type instabilities and that there is only one gap in the otherwise continuous plasma cylinder. The gap in the plasma is adjacent to the power feed to the theta piece so that it may be ignored as a wedge effect. Since there are no apparent Rayleigh–Taylor instabilities in Figs. 5 and 6 we assume that the radial velocity of the plasma is constant and that the acceleration required for the growth of instabilities is only present for a very short time during the initial formation of the imploding cylindrical shock. If the current sheet had accelerated for a longer time we would have expected to see the same type of instabilities as those observed by Niblett and Green[1].

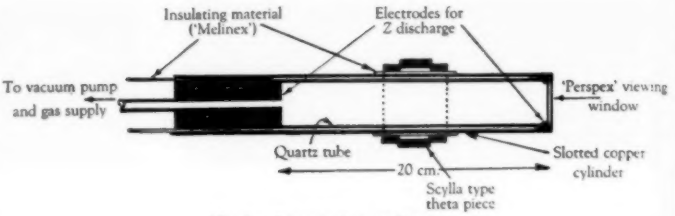

Fig. 8. Cross-section of *Prize*

Our experimental evidence suggests that the radial wedges are only formed when there is a trapped magnetic field in the same direction as the driving field, that is, when the azimuthal currents on the shock front and the current sheet have opposite directions of rotation. This hypothesis is also supported by the Niblett–Green[1] photographs, which show that radial 'wedges' are present when there is parallel trapped field. A possible reason for this is as follows: if the currents are in opposite directions Rayleigh–Taylor instabilities on the current sheet will produce flutes which can grow until they break up the plasma into radial wedges, each wedge carrying its own circulating current. The wedges travelling inwards at shock speeds produce bow waves which fill the interstices between the wedges with less highly ionized plasma. If the trapped field is in the opposite direction to the driving field the currents are in the same direction and the flutes produced by the instabilities in the current sheet are unable to break up the plasma, constrictions similar to the sausage type $m = 0$ instability observed by Josephson should then occur. An attempt to show the two effects graphically is made in Fig. 7.

Recent experiments on the *Scylla* apparatus[5], with the deuterium gas pre-ionized so that neutrons were observed in the first compression half-cycle, have shown that the neutron output was greatly enhanced by the inclusion, prior to the discharge, of a small axial magnetic field in the direction opposite to the compression field. We think there may be a connexion between this recent discovery and the instability mechanism discussed above.

We wish to thank Dr. R. Latham of the Imperial College of Science and Technology and Drs. H. R. Hulme and N. J. Phillips of the Atomic Weapons Research Establishment, Aldermaston, for stimulating discussions, and Messrs. A. Skinner and R. J. Abrey for the loan of their high-speed framing camera and their help and guidance in its use.

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A NEW TEST FOR DETECTING ANAPHYLACTIC SENSITIVITY: THE BASOPHIL REACTION

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EACH year many individuals in seeming perfect health meet sudden death as a result of anaphylaxis. Such overwhelming allergic reactions reflect an immediate type hypersensitivity to diverse drugs, foods, insect bites or indeed even cold. Of all these, penicillin is perhaps the most common cause of anaphylactic shock and death. Although much is known of anaphylaxis, no safe reliable predictive test is yet available. Skin tests (direct, passive, and passive cutaneous anaphylaxis), serological antibody studies, as well as animal smooth muscle reactions each suffer serious limitations in clinical practice.

In our attempt to develop a test for anaphylactic reactors, two well-documented observations appeared to be of signal interest. First, all the key signs of anaphylaxis are, or may be, induced by histamine. Secondly, the depot of histamine in the blood is the basophilic granulocyte[1]. Thus, it seemed likely to us that in anaphylaxis the basophil might release its stores of histamine, with consequent degranulation similar to that observed in its congener, the mast cell[2]. Transmutation of this idea into experimental data has required two years for developing a technique to handle and to concentrate this at once fragile and rare white cell of the blood. We are now able to

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examine a miniature *in vitro* anaphylaxis system consisting of the human basophil, blood and antigen. Such work reveals the basophil to be a cell with a cytological picture reflecting its functional state. Hence it can serve as a laboratory index of anaphylactic sensitivities. The following is a preliminary account of our results.

The majority of these observations were made on twenty consecutive penicillin-sensitive patients and an appropriate twenty non-sensitive healthy control subjects. Limited investigations were also performed on four additional patients who had experienced severe systemic reactions to cold, bee stings and prochlorperazine. The following general sterile technique was employed. Tests were not made on subjects after a fat meal, during corticosteroid therapy, systemic illness, urticarial or anaphylactic reactions. At such times the basophils were often inadequate for testing purposes. Specimens of venous blood (0·25 ml., 5 μgm. heparin sodium added), drawn with siliconized needle and syringe, were mixed ('Vortex Jr.' mixer) with 0·025 ml. of penicillin or appropriate antigen in a concentration of 10^{-3} in physiological saline solution. After standing in a polystyrene tube at room temperature (25° C.) for 15 min., 0·1-ml. samples were withdrawn in siliconized syringes and added to ice-cold fixative solution (ethyl alcohol 6 ml., chloroform 2 ml., and acetone 2 ml.). After overnight fixation, at +4° C. the cells were re-suspended and aliquots were filtered through a cellulose membrane filter (Schleicher and Schuell, coarse 'Cella *O*', 20 min.). The filter was then selectively stained with toluidine blue (30 sec., 1/300 aqueous, phosphate buffer *p*H 5·2) dehydrated in ethyl alcohol and cleared in xylene. After mounting, the basophils could be clearly seen, counted, typed and photographed. Routinely a standard count of 20 consecutive basophils was made. The basophils were classified into distinct categories on the basis of size, staining properties, number and location of basophilic granules. Such a 'basophil differential' permitted accurate assessment of the effect of exposure to given antigens. In essence, the technique enabled us to distinguish the depleted from the intact cell. Depleted cells appeared as basophils (*a*) with few granules, or (*b*) with small lightly stained granules, or (*c*) with an appreciable number of granules seen outside the cell. The preparations were deemed unsatisfactory when the control slide showed more than one-fourth of the cells 'fired'. Our initial investigations suggest that for a positive test at least half the basophils exposed to the test antigen should show evidence of degranulation (depletion). It is apparent that test antigens must not be intrinsically 'histamine liberators' and must be used in concentrations which have no effect on the granulation of basophils in normal controls.

Table 1 reveals that each of the five patients with a history of penicillin anaphylaxis showed significant degranulation of the basophils in the presence of penicillin *in vitro*. It is important to note that the episodes of anaphylaxis predated the testing by months, even as long as nine years. Thus, anaphylactic penicillin sensitivity may be remarkably persistent. In the fifteen who gave a history of urticaria associated with penicillin (various types and routes of administrations, over a ten-year period), five had a positive test as defined by us. This urticarial group is heterogeneous. Some members may be sensitive to procaine or other substances, rather than penicillin. Others may have lost their sensitivity over the years,

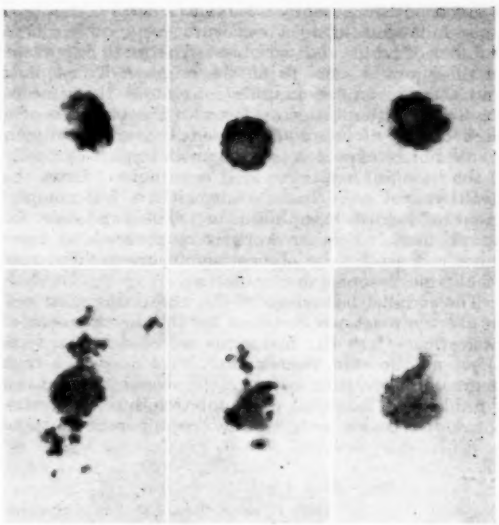

Fig. 1. Human basophils observed in an *in vitro* test for anaphylactic sensitivity. Upper three control cells show relatively intact structure. Lower three cells show varying degrees of explosive degranulation. The test is positive when more than 50 per cent of the cells show this depleted appearance after 15 min. exposure to test antigen in 10^{-4} concentration. (× 1,750)

and, although re-administration of penicillin might re-light it, they are nevertheless non-sensitive at the time of our testing. None of the controls was positive. This is significant since these individuals had each had penicillin without any clinical reaction. Some were actually taking it at the time of testing. It would seem desirous to correlate further these results of predictive testing of penicillin sensitivity with the clinical administration of penicillin. This may be done in animal work, but not in man because of the potentially grave consequences. We can rely at present only on the close correlations of history and *in vitro* studies.

Table 1 further shows that the results of the basophil test correlate well with the clinical histories of individuals who have experienced major or minor anaphylaxis as a result of immersion in cold water, yellow jacket bee sting, as well as another drug hypersensitivity, namely, prochlorperazine.

We feel that the test described aids in the detection of the anaphylaxis-prone individual. Nevertheless, negative data in this test reflect the patient's status only at the time of testing and do not anticipate any possible future sensitization. The test gauges the critical potential for anaphylaxis by determining the degree of anaphylactic sensitivity. Whether or not

Table 1

Clinical history	Antigen (10^{-4}) tested	No. of patients	Positive reactors
Penicillin anaphylaxis	Benzyl penicillin potassium	5	5
Penicillin urticaria (presumptive)	Benzyl penicillin potassium	15	5
Normals	Benzyl penicillin potassium	20	0
Cold anaphylaxis	+ 4° C. bath	2	2
Normals	+ 4° C. bath	10	0
Prochlorperazine urticaria	Prochlorperazine	1	1
Normals	Prochlorperazine	3	0
Yellow jacket Bee sting Anaphylaxis	Yellow jacket extract	2	2
Normals	Yellow jacket extract	2	0

a given patient actually experiences clinical anaphylaxis following antigen exposure rests on a large number of additional variables. The most important of these would seem to be the number of basophils and their histamine granule content at the time of exposure to the antigen. Even in the presence of a high degree of anaphylactic sensitivity, an antigen would not be expected to have much impact clinically if the basophil histamine load were light. Thus, the healthy, yet sensitized, adult with a full complement of loaded basophils is a prime candidate for anaphylaxis. Further work is in progress to learn more concerning the clinical significance of the rate of allergic basophil degranulation.

The parallel behaviour of the cutaneous mast cell in allergic reactions accounts for the development of urticaria. Here the histamine released has a local effect on the skin vasculature[3]. We suspect a high degree of correlation between the release of mast cell granules and basophil degranulation, but the dichotomy of the two systems may be apparent at times and thus account for negative skin tests in a known anaphylactic reactor.

'Basophil anaphylaxis' affords new cyto-immunological vistas since this target cell reaction can be observed in animals as well as in man. The data presented at least suggested that the basophil is the central cell of the anaphylactic state. It is hoped that the new technique described here will permit an intensive study of this heretofore elusive and labile cellular rarity of the blood stream.

This work was conducted under the sponsorship of the Commission on Cutaneous Diseases of the Armed Forces Epidemiological Board and was supported in part by the Surgeon General, Department of the Army, and in part by grant *PHS 2G-62* from the National Institutes of Health.

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STEROIDS AS ICE NUCLEATORS

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MOST substances suggested as possible ice nuclei for cloud seeding are inorganic compounds of hexagonal structure with lattice parameters similar to those of ice. The possibility of using organic compounds as ice nuclei has not yet been explored. During an investigation of water-insoluble organic compounds, seven out of thirty steroids examined, the symmetry of which was mainly monoclinic or orthorhombic, were found to nucleate ice almost as effectively as silver iodide. The discovery of a class of organic materials capable of acting as ice nuclei appears to be novel.

Activities were determined by means of a microscope cold stage into which slides carrying steroid particles could be inserted. Provision was made for the introduction of moist air. The particles under test thus acted as freezing nuclei.

Although nucleations occurred on a proportion of crystals slowly grown from alcohol, as shown in Fig. 1 (androstenolone at $-12°$ C.), none occurred on small needles or plates grown rapidly. Particularly active sites were engendered by quick recrystallization

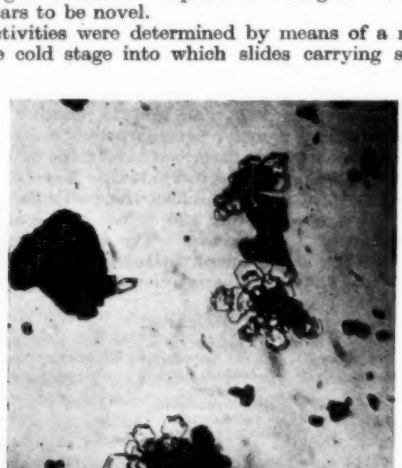


Fig. 1. Ice crystals growing on androstenolone at $-12°$ C.

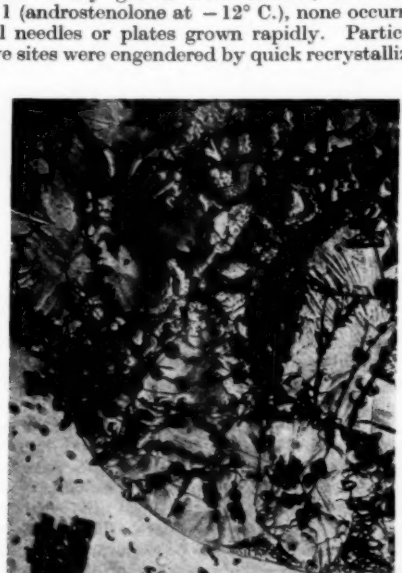
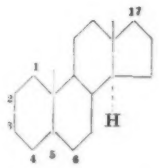


Fig. 2. A recrystallized droplet of androstenolone showing multiple nucleations on cracks

at the melting point in air or pure nitrogen, followed by quick cooling. This was done by heating a slide, powdered with crystals, at one end until about half the particles had melted. A line of particles which had recrystallized near the melting point was thus formed at the junction of the melted and unmelted portions, and these, together with the larger droplets which had recrystallized on cooling, showed multiple nucleations, especially at cracks. Fig. 2 shows part of a recrystallized droplet of androstenolone on which more than a hundred nucleations occurred at $-7°$ C. Droplets smaller than about 0·03 mm. diameter were usually inactive.

The most active steroids were androstenolone (I), testosterone (II), 3α-methylcholestan-3β-ol (III), androsterone (IV), pregnenolone (V), androstan-3,17-dione (VI), and cholesterol (VII), for which the onset temperature was $-5°$ C. Nucleation of all particles larger than 0·03 mm. diameter was complete at $-7°$ C.



Compound	Position of C=C	Configuration at position 5	Substituent at C_3	Substituent at C_{17}
I	5–6	—	β-OH	=O
II	4–5	—	=O	β-OH
III	—	5α-H	αMe-β-OH	β-C_8H_{17}
IV	—	5α-H	α-OH	=O
V	5–6	—	β-OH	β-$COCH_3$
VI	—	5α-H	=O	=O
VII	5–6	—	β-OH	β-C_8H_{17}

Much lower activities were shown by androsta-1,4-dien-3,17-dione, cortisone, cholestenone, progesterone and œstrone, while no activity was shown by steroid esters, ergosterol, 17α-methyltesterone or 3β-methylcholestan-3α-ol, although the 3β-ol compound was very active. Substances with no groups capable of hydrogen bonding such as 3β-methylcholestane were inactive.

It is usual to associate nucleation with the similarity in spacing between the ice lattice and that of the nucleating substance. The exact crystal structures of many steroids are still unknown; but the survey by Bernal, Crowfoot and Fankuchen[1], and the detailed analyses of cholesteryl iodide[2], and lanostenyl iodoacetate[3], suggest that the molecules in the majority of steroidal structures are arranged in parallel stacks with 'head-to-tail' packing. One surface of the stack would thus present a pattern of alternating hydroxy groups and side-chains. It is presumed that nucleation takes place on this surface. The relationship between the cell dimensions of ice and those of the steroids is not obvious, but the cross-sectional dimensions of the steroid molecule, 5 ± 1 Å. × 7 ± 1 Å., deduced by Bernal *et al.*[1] suggest a possible compatibility with the prism face of ice (4·51 Å. × 7·35 Å.) although it is realized that this spacing is only relevant if the molecules are stacked approximately normal to the surface. The precise measure, however, would depend on the structural dimensions of the crystal form.

It has been shown that the most active substances have keto or hydroxy groups at position 3, and it is notable that three of them have keto groups at position 17 in addition. That these groups may be interchanged without loss of activity is illustrated by the comparison of androsterone versus testosterone. If it is assumed that the essential requirement for activity is a hydroxy or keto group at position 3, it is clear why no activity is shown by the steroid esters since esterification occurs at this position. The failure of 3β-methylcholestan-3α-ol and 17α-methyltestosterone to show activity is probably due to the shielding of axial hydroxy-groups by equatorial methyl groups.

It is interesting to find a class of compounds in which the presence of nucleation sites depends so much on the physical methods of sample preparation. The most active samples of cholesterol prepared appear to contain robust sites which will withstand radiation equivalent to a 100 min. of sunlight without losing activity. This may be due to the inaccessibility of other positions of attack if only the edges of the molecular stack are exposed.

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IDENTITY OF L-ASCORBIC ACID FORMED FROM D-GLUCOSE BY THE STRAWBERRY (*FRAGARIA*)

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WORK in this Laboratory has shown that ascorbic acid recovered from ripening strawberries or germinating cress seedlings administered D-glucose-1-^{14}C is labelled primarily in carbon 1 (the carboxyl carbon)[1,2]. In these experiments, ascorbic acid was removed from the plant extract with an ion exchange resin, 'Dowex 1-formate', selectively eluted by means of a formic acid gradient, diluted with non-isotopic L-ascorbic acid, recrystallized from methanol and ethyl ether mixtures or from glacial acetic acid until there was little or no change in specific activity, and, finally, degraded chemically to determine the specific sites of ^{14}C labelling. A portion of the ^{14}C-containing ascorbic acid plus carrier L-ascorbic acid was oxidized with *p*-quinone to dehydroascorbic acid and then converted to its di-*o*-phenylenediamine derivative[3]. The free acid of this derivative, crystallized from water, had the same specific activity as the ascorbic acid. Conversion of this free acid to its lactone and

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recrystallization from methanol also yielded a product with the same activity.

When these results were published it was felt that these observations sufficed to establish the exact isomeric nature of the labelled ascorbic acid from the strawberry or the cress seedling as L-ascorbic acid. However, Takahashi, Mitsumoto and Kayamori recently published two notes[4] describing the formation of D-araboascorbic acid (D-*iso*ascorbic acid) by various species of the mould, *Penicillium*, when it was grown on D-glucose, D-gluconic acid or sucrose. They also found that *P. notatum* converted D-glucose-1-^{14}C to D-araboascorbic acid in which 80 per cent of the ^{14}C of the molecule was located in carbon 1. The discovery that *Penicillium* can convert D-glucose to D-araboascorbic acid suggests that a more critical appraisal should be made of the isomeric nature of ascorbic acid recovered from higher plants which have been administered specifically labelled D-glucose-^{14}C.

This communication presents the results of an experiment designed to compare L-ascorbic acid and D-araboascorbic acid as carrier substances in the successive recrystallization of labelled ascorbic acid obtained from a ripening strawberry administered D-glucose-6-^{14}C. D-Glucose-6-^{14}C (50 μc. at 7·7 mc./m.mole) was administered in 0·1 ml. of water through the cut stem of a strawberry in the green–white stage of ripening. It required 3 hr. for the aqueous solution to be taken up by the strawberry. After an additional 40 hr. with the stem immersed in distilled water, the fruit (less hull and stem) was frozen, mascerated in ethanol at − 40° and the ascorbic acid recovered without carrier dilution on a 'Dowex 1-formate' exchange resin. The ascorbic acid was selectively eluted with a gradient of 0 → 0·06 *N* formic acid and the fractions containing ascorbic acid, as characterized by titration of aliquots with 2,6-dichlorophenol indophenol, were pooled. These pooled fractions, which contained 5·6 mgm. of reducing material, were divided into two equal portions. To one was added 200 mgm. of D-araboascorbic acid and to the other, 200 mgm. of L-ascorbic acid. Each was evaporated to a syrup, taken up in glacial acetic acid and allowed to crystallize in the cold. Subsequent crystallizations were also done in glacial acetic acid. The radioactivities of each series of crystallizations are summarized in Table 1. With L-ascorbic acid as the carrier, the product after three crystallizations was only slightly less active than after the first crystallization. By contrast, nearly all the carbon-14 remained in the mother liquor when D-araboascorbic acid was used as a carrier. Less than 2 per cent of the specific activity found in the portion diluted with L-ascorbic acid was retained in the portion diluted with D-araboascorbic acid.

The identity of labelled ascorbic acid as L-ascorbic acid was also confirmed by preparing two derivatives of the product representing the third crystallization of the L-ascorbic acid-diluted portion and by comparing their activities with the original ascorbic acid. Both derivatives had specific activities similar to the ascorbic acid used in their preparation (Table 1). Derivatives of the carrier L-ascorbic acid and D-araboascorbic acid were also prepared and submitted to microanalysis.

The 5,6-*iso*propylidene L-ascorbic acid was prepared by the method of von Vargha[5] and recrystallized from absolute ethanol. Calculated for $C_9H_{12}O_6$: C, 50·0 per cent; H, 5·60 per cent. Found: C, 50·1 per cent; H, 5·67 per cent. Attempts to prepare 5,6-*iso*propylidene D-araboascorbic acid by the same procedure resulted in a product having the same melting-point and elemental analysis as D-araboascorbic acid and showing no melting-point depression when mixed with authentic D-araboascorbic acid. Calculated for $C_6H_8O_6$: C, 40·9 per cent; H, 4·57 per cent. Found: C, 41·0 per cent; H, 4·71 per cent. Apparently, the conditions sufficient for the formation of 5,6-*iso*propylidene L-ascorbic acid cannot effect the formation of 5,6-*iso*propylidene D-araboascorbic acid.

Table 1. Radioactivity of Carrier-diluted Ascorbic Acid from a Strawberry Labelled with D-Glucose-6-^{14}C

Diluent	L-Ascorbic acid		D-Araboascorbic acid	
	Amount recovered (mgm.)	Radioactivity, mμc./mmole of ascorbic acid	Amount recovered (mgm.)	Radioactivity, mμc./mmole of ascorbic acid
First crystallization	155	74·7	97	8·2
Second crystallization	99	68·7	79	2·0
Third crystallization				
First crop	48	66·5	56	1·0
Second crop	12	69·2		
5,6-*Iso*propylidene derivative		57·4*		
2,3-Diphenacyl derivative		64·2*		

* Activities corrected for a 5·76-fold dilution in the case of the diphenacyl derivative and an 11·7-fold dilution in the case of the *iso*propylidene derivative. These derivatives were wet combusted with Van Slyke–Folch reagent to carbon dioxide and counted in an internal gas phase proportional counter (ref. 14). Other activities were determined in a liquid scintillation apparatus using a liquid scintillation mixture of 0·6 gm. naphthalene, 14·5 ml. *p*-dioxane containing 0·4 per cent 2,5-diphenyloxazole and 0·01 per cent 1,4-*bis*-2(5-phenyloxazolyl)-benzene and the radioactive ascorbic acid in 0·5 ml. of water (ref. 15).

A second derivative, 2,3-diphenacyl L-ascorbic acid[6], was prepared according to the same procedure used to obtain diphenacyl malate[7]. The crude, moist, crystalline product was recrystallized immediately from ethanol in order to avoid the partial decomposition otherwise observed. Calculated for $C_{22}H_{20}O_6$: C, 64·1 per cent; H, 4·88 per cent. Found, L-isomer: C, 64·1 per cent; H, 5·02 per cent. M.p. 137–138°. D-isomer: C, 63·2 per cent; H, 4·99 per cent. M.p. 139–140°.

A portion of the recrystallized L-ascorbic acid diluted fraction was further diluted with authentic L-ascorbic acid (non-isotopic) and degraded[8,9], and the carbon-14 distribution results are given in Table 2. This pattern confirms one obtained previously from a strawberry labelled with D-glucose-6-^{14}C (ref. 10).

Table 2. Distribution of Carbon-14 in L-Ascorbic Acid-diluted Portion after Three Crystallizations

	Radioactivity*		Total ^{14}C (per cent)
	mμc./m.mole of carbon	mμc./m.mole of ascorbic acid	
Carbon 1 + 2	6·14	12·28	18
Carbon 3	5·25	5·25	8
Carbon 4 + 5	0·86	1·72	3
Carbon 6	47·18	47·18	71
Sum of degraded fragments		66·43	
Van Slyke–Folch wet combustion	11·08	66·48	

* Activities of the degraded fragments are corrected for a final 5·35-fold dilution of L-ascorbic acid after the third crystallization. All counting data were obtained with an internal gas phase proportional counter (ref. 14).

but more importantly, it provides a definitive reply to suggestions[11,12] that such a pattern could also be obtained if D-glucose-6-^{14}C were converted by two separate pathways to a mixture of L-ascorbic acid (labelled primarily in carbon 1) and D-araboascorbic acid (labelled primarily in carbon 6). The present results show that 71 per cent of the carbon-14 in the L-ascorbic acid remained in carbon 6, whereas the residual activity found in the D-araboascorbic acid after two recrystallizations may have been due to traces of L-ascorbic acid.

It may be concluded from this brief study that higher plants do contain an enzymic process capable of converting D-glucose to L-ascorbic acid over a pathway which preserves the carbon skeleton and does not invert the carbon chain yet does result in an epimerization at carbon 5 from the D to the L configuration. Tracer work on ascorbic acid biosynthesis in plants preceding the experiment just described has been reviewed in a paper that will be published shortly[13].

We acknowledge the help of Miss Geraldine Secor, who performed the microanalyses, and the suggestions of Dr. Peter Dayton, Goldwater Memorial Hospital, New York, concerning the preparation of 5,6-*iso*propylidene L-ascorbic acid.

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AMINO-ACID LIBERATION FROM PROTEINS BY ENZYME OR 'HEAT CATALYSED' HYDROLYSIS

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THE term 'heat catalysed' hydrolysis is introduced here for the first time to emphasize a certain phenomenon in the so-called acid/alkali hydrolysis of proteins which has been overlooked apparently or underestimated hitherto. The term is used to point out some interesting similarities between acid/alkali and enzymic hydrolysis, such as the similar parts played by the enzyme and by the heat in bringing about the liberation of amino-acid from the peptide chain. At the same time certain dissimilarities between the two processes will be indicated, and it will be shown how these may explain the significant differences in the physical parameters of the resultant amino-acids.

Analysis of urinary peptide and amino-acid excretion in children has been carried out recently using a high-tension electrophoretic apparatus which I have designed and constructed. This was of the free-floating type. It was kept at a constant temperature of 0° C. by a refrigerated *n*-hexane circulating system controlled by thermistors. The cooling was efficient enough to maintain the entire length of the electrophoretic paper at within 0·5 deg. C. of the same temperature. This was checked by the use of a series of ten specially designed thermometers which were placed in contact with the paper at 10-cm. intervals. Thus the temperature of the paper was measured throughout the entire operation.

Whatman No. 4 paper 1 cm. wide and 120 cm. in length was used. The paper was suspended horizontally through the centre of the cooling liquid with the nylon-sheathed ends dipping into the two buffer-electrode containers. The buffer used had a *p*H of 1·9 (acetic acid–formic acid–water, 3 : 1 : 16) with a conductivity of $0{\cdot}3 \times 10^4$ micro-ohm. The power used was 200 V./cm. length of paper and thus 1·0 m.amp./cm. width of paper for 25 min. was produced. While this was taking place several hitherto undescribed phenomena in the behaviour of amino-acids at high field strengths were observed.

Among these was one which initially caused some difficulties, when it was attempted to identify the separated fractions by adding, to the ion-exchange resin-desalted urinary sample, commercial amino-acids of high purity which were known by previous chromatographic tests to be present in the urinary sample. The result was that these added amino-acids, instead of joining up with their urinary counterparts, and thus increasing the intensity of the subsequent colour reaction, produced separate additional fractions on their own.

The findings of an experiment in which three commercial amino-acids were added to the ion-exchange resin-desalted urinary sample are demonstrated in Fig. 1. The original urine of a healthy child yielded, according to previous chromatographic evidence, six amino-acids in ninhydrin-staining quantities; the same six amino-acids were also found to be separated on subsequent high-tension electrophoretic testing. Strip *A* represents the urinary sample, yielding aspartic acid, proline, glutamic acid, threonine, serine and glycine. On strips *B*, *C* and *D*, aspartic acid, threonine and proline were added to the urinary sample respectively. Finally, on strip *E* all three commercial amino-acids that were added can be seen in a simple buffer solution alone.

It is apparent that in no case have the added amino-acids joined up with their urinary counterparts. They have produced instead an additional fraction on their own account which is clearly identifiable.

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XUM

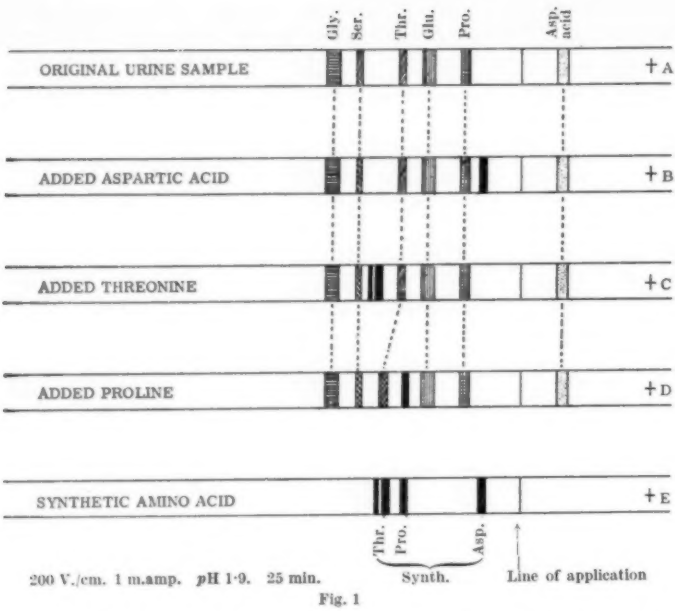

Fig. 1

(Threonine has produced not one, but two fractions on strip *C*, as well as on strip *E*. This is thought to be due to the fact that this amino-acid is a racemic mixture of D- and L-threonines. This particular finding and the possible explanation for it will be discussed separately in a later paper.)

This test was repeated chromatographically, but no such behaviour was then observed. The commercial amino-acids which were added merely joined up and increased the ninhydrin colour reaction of the appropriate amino-acids.

After repeating these tests and obtaining the same results, it was concluded that the underlying explanation for this specific and unexpected behaviour must lie in the electric parameters of the respective amino-acids, since it was found to manifest itself only when they were subjected to a strong electric field.

The major factor determining the mobility of amino-acids in electric fields is the molecular electric charge of the amino-acid itself. It is assumed, therefore, from the findings described here that the molecular electric charge of urinary and commercial amino-acids must differ.

The literature has been searched for descriptions of similar experiences by others using high-tension electrophoretic techniques. It was found that the actual number of authors who describe their results in detail using various means of chemical identification of the separated fractions is very small, and that those who do so do not mention such difficulties as have just been described.

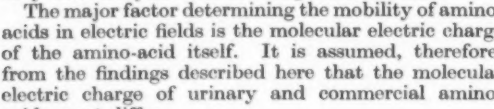

Fig. 2

In one single instance, however, in an article by Heilmeyer *et al.*[1], a photograph of an original high-tension electropherogram was found (Fig. 2). This illustration (Fig. 3 of the original article) shows two stained high-tension electropherograms. On the upper strip four amino-acids separated from the resin-desalted urine sample can be seen. Underneath is another strip, on which it can be seen that the actual number of separated fractions has increased by two. The caption states that in the case of the lower strip two commercial amino-acids, cysteine and glycine, have been added (these are marked Cy and Gly). However, there is no mention, in either the text or caption, of the fact that the number of separated fractions has increased. This increase in this case is thought to be a confirmation of my own findings.

The importance of this specific behaviour of amino-acids, the fundamental building blocks of proteins, in electric fields will become more evident if we consider that, in biological circumstances in living systems, proteins, peptides and amino-acids, as well of course as other metabolites, are under the permanent stress of electric fields applied across biological membranes. These electric fields are maintained by the difference in concentration of the electrolytes on the two sides of the membrane, and they amount to an average potential difference of 100 mV. Since the average thickness of such membranes is of the order of 10^{-6} cm., the average actual value of these biological membrane potentials is of the order of 100,000 V./cm. Thus it can be said that the electric field maintained by my high tension electrophoresis is somewhat sub-biological, being about a tenth of the average of the living cell. These biological potentials, however, are characteristic and inherent parts of the living systems and their changes are the foundations of life.

The importance of this biological electrical field has already been emphasized in a previous report[2]. Attention was directed to the fact that there may be a selective biological membrane transport of individual amino-acids due to these biological potentials.

It would therefore seem important to try to elucidate the real foundations of this unexpected behaviour of amino-acids derived from different modes of liberation.

Living organisms liberate these amino-acid metabolites from food proteins by the use of enzymes at the average body temperature of the living species. The amino-acids that are commercially available, on the other hand, are either liberated by acid/alkali or enzymatic hydrolysis of a readily hydrolysable protein, for example, casein, or else are the result of bacterial metabolic production. It was thought that a closer investigation of at least the two main types of liberating processes—the acid/alkali and the enzymic—might provide some explanation as to why commercial amino-acids bear a different molecular electric charge from their urinary counterparts.

In the enzymic hydrolysis of proteins, the protein substrate is first subjected to an adjustment of its original *p*H. This will be according to the optimum range of *p*H for the enzyme(s) to be used. This imposed change of *p*H can be either on the acid or on the alkali side. The importance of this *p*H adjustment is obvious from theoretical considerations, and it was assumed earlier that the carriers of catalytic activity of enzymes were mostly the zwitterionic forms of the molecule, since it was observed that in most cases the optimum *p*H coincided with the isoelectric point of the enzyme. This was first suggested by Michaelis and Davidsohn[4], but since then it has been discovered that this is often not the case, and the isoelectric point does not really coincide with the optimum *p*H; therefore, it is supposed that the activity is dependent on the ionization of certain groups on the enzyme molecule and not always necessarily in the zwitterionic form.

The importance of the effect of *p*H on enzymic activity from the point of view of the quality of hydrolysis is laid down in an excellent work by Wilson and Nachmanson[5], in which they elucidated the reaction mechanism of the acetylcholinesterase enzyme. Waley[6] and Dixon[7] have demonstrated the effect of *p*H in general on enzymic activity.

After the adjustment of the *p*H of the protein substrate, the chosen enzyme(s) is introduced, and at the optimum temperature, the process of enzymatic hydrolysis proceeds.

Since it is proposed to deal later on with the heat-catalysed acid/alkali hydrolysis of proteins, it is thought that the meaning of the term 'optimum temperature' should be looked at more closely. Temperature is surely one of the most influential and important factors governing reaction mechanisms specifically in biological processes. The optimum temperature is generally considered to be the deg. C. at which the maximum rate of reaction occurs, or in other words, at which the enzyme will liberate the greatest number of amino-acids from the protein substrate in a given time. As early as 1895, Tammann[8] suggested that enzymic processes show acceleration when the temperature is raised, but when a maximum point has been reached will slow down rapidly again on account of thermal destruction of the enzyme protein molecule itself, in which case irreversible changes take place in the molecular configuration of the enzyme which greatly affect its specific activity, and finally arrest it. It will be seen that this optimum temperature is a range of temperatures rather than a sharp point, since living systems are accustomed to certain degrees of change in their environmental temperature, which in turn influence their internal temperature; thus, their metabolic processes have acquired, during the course of evolution, a certain flexibility in their response to changes in temperature over a limited range. It is important to maintain the optimum temperature in enzymic processes, because if it falls or rises the inherent energy content of the reactive system would be either reduced or increased, thus causing variation in an important factor in the reaction mechanism.

It can be said with certainty at this stage that in enzymic hydrolysis, whether *in vitro* or *in vivo*, change of entropy does not occur appreciably; no mass resonance, rotation, translation or vibration of the electron system of the reacting molecules occurs and the force binding the enzyme to the substrate protein molecule acts by straightforward contact and is dependent among others on intermolecular distances. Charged groups on the substrate molecules become attached to groups on the enzyme molecules bearing opposite charges. This bond may be analogous to 'salt-bridge' formation in proteins. It is known that the K_S (dissociation constant of the enzyme-substrate complex) is infinite only for positively charged substrates of trypsin. Hydrogen bridges may also play a part. Protein molecules are capable of forming intramolecular hydrogen bridges, and with a suitable substrate it is plausible that intermolecular hydrogen bridges can also be formed. This is thought to be the case with the proteases. Van der Waals forces also come into play, for example, in the action of cholinesterase, where the K_S decreases and therefore the affinity increases with the increasing length of the hydrocarbon chain in the acyl esters of choline. Finally, it should be mentioned that the substrate can also react with the enzyme in the formation of covalent bonds.

In all these types of enzymic hydrolysis reactions the reacting substrate protein molecules utilize their molecular free-energy content, for no external energy is supplied to them in any form; the maintenance of a constant optimum temperature prevents them from losing energy unproductively.

It is also well known that enzymic hydrolysis of proteins can be repeated successfully with the same results so long as the delicate experimental conditions are adhered to strictly. The specific mode of action of the various enzymes has been described and explained by Neurath and Schwert[9], Smith[10] and Green and Neurath[11].

In the heat-catalysed hydrolysis of proteins the substrate protein undergoes the same preliminary treatment as in the enzymic process described above, namely, its *p*H is changed. But in this case, instead of introducing the enzyme into the *p*H-adjusted substrate, external energy is supplied in the form of excessive heat, and thus amino-acid liberation occurs.

A well-known phenomenon in chemistry is the increase in the rate of reaction which occurs when the temperature of the reactive mixture is raised. This increase cannot be accounted for solely by the increased number of molecular collisions because, as a rule, the rate of the reaction increases with the rising temperature far more sharply than does the frequency of the collisions. It will be remembered that reacting molecules having an average velocity do not generally produce chemical reactions since they do not have enough energy to develop into the transitional state. This transitional state, from the point of view of energy consumption in reactive phases, is the top of a curve up which the reacting molecules must have sufficient energy to climb before they can descend down the other side, releasing energy and forming the product. The rate of reaction is related then to the number of molecules that pass from the reactive side to the productive side of this curve in a given time. Raising the temperature will necessarily increase the number of molecules with sufficient energy to take part in this process.

The thermodynamic constants, for example, heat, entropy, etc., of activation and reaction supply a good picture of the stages of reaction in these cases. Kunitz[12] has provided evidence for the reversible denaturation by heat of crystalline trypsin inhibitor, a globular protein of 25,000 mol. wt. He found that when the native protein was denatured reversibly, the heat generated by the reaction was 57·3 k.cal./mole and the entropy change was 180 cal./°C./mole. There was a great increase in entropy and no increase in the number of molecules in the substrate heated.

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This indicates that the denatured form of the molecule is formed from the native state by bond breakage, and thus it has greater freedom of movement than before. It is an interesting point that the total energy of the activated complex is almost the same as that of the denatured form and very different from that of the native form.

This activated complex is an intermediate form of the native molecule in its progression to the end product and its lifetime is about 10^{-13} sec. Once formed, it decomposes with a frequency of kT/h, where T is the absolute temperature, h is Planck's constant and k is Boltzmann's constant, that is, R/N the gas constant per molecule. The precise significance of the activated complex, however, can only be appreciated by extensive reference to the quantum theory and statistical methods, subjects which it is not intended to develop further here.

However, the entropy of the activated complex is still much less than that of the total completed reaction, that is, the sum of the entropies of the amino-acids liberated. Under the compelling influence of the constantly available supply of excess energy the activated protein molecule, while not gaining in its molecular free-energy content, steadily increases its entropy until such a state of instability is produced that some of its valence bonds will be broken. The remaining bonds will break spontaneously as time progresses.

It is reasonable to assume that the excess energy in the activated protein complex at the time of its final break-up will reside in the resultant amino-acids.

It has been found by Eyring and Stearn[13] using trypsin, and by Eisenberg and Schwert[14] using chymotrypsin, that the value of the thermodynamic constants of heat denaturation of proteins depends significantly on the *p*H of the substrate. This is interesting if it is considered together with the dependence of *p*H of the enzymic activity.

The fact that it has many drawbacks is a well-known characteristic of such a heat-catalysed liberation of amino-acids. These include structural modification, racemization, tautomerization, destruction and artefact production of various types of amino-acids, as have been described by Neuberger[15], Spies and Chambers[16], Dakin[17], Levene and Steiger[18], and Sanger and Thompson[19]. These authors are all in agreement, and they suggest that such amino-acids as are formed during heat-catalysed hydrolysis of proteins are chemically and biologically identical to those liberated by enzymatic hydrolysis. They admit that side-effects are unavoidable, and although they are to some extent undesirable, they account merely for some quantitative and qualitative loss or gain of some specifically known amino-acid.

In attempting to find the fundamental difference between enzymic hydrolysis or enzyme-catalysed hydrolysis and acid/alkali hydrolysis or heat-catalysed hydrolysis, it becomes apparent that it is to be looked for in the very similarity of the two methods. The two kinds of liberating agents, the heat and the enzyme, both produce the same result, namely, bringing about breakage of the terminal bonds of amino-acids: without them there would be no significant liberation of amino-acids. But if we go one step further and consider the way in which these two agents, to such an extent similar, achieve their final target, we must realize the essential dissimilarity between their activities.

What kind of factor is it then which can be pointed out as responsible for the dissimilarities in the molecular electric charges of the liberated amino-acids, since they are chemically and probably, so far as we know at present, biologically identical? It is unlikely to be found in the similarities between the two methods. But in considering the differences between them, in terms of the utilization of energy, exchange of energy and the general relations between the energies involved in the processes, the real cause may be brought to light.

In enzymic processes, as I have already pointed out, there is no need whatever to provide energy externally, as there is in heat-catalysed hydrolysis. All the energy needed to proceed from one side of the 'hill' over the 'summit' of the intermediate complex to the resultant reactive side of the liberated amino-acids is obtained from the molecular free-energy content of the protein substrate itself, and of the enzyme without significant change in the entropy of the system and well within the limits of thermodynamics. This is why any kind of gross structural irregularity, artefact production or destruction of amino-acids during enzymic hydrolysis would not be expected. Everything proceeds along a fairly well-determined pathway in stages which can to some extent be calculated from the thermodynamic equations.

But this is not the case in heat-catalysed hydrolysis. In this method of liberation of amino-acids, excess energy is transferred to the protein substrate. An unlimited amount of energy is available for any type of energy-consuming reaction to take place. The intra- and inter-molecular bonding electrons of the protein substrate molecules are also excited by means of resonance to extreme levels. The heat energy is absorbed by the electron system and transformed into electrical form. The molecular electric charge of the resultant amino-acid ultimately depends on the behaviour of the electron pool. These electric effects are not great enough to cause alternations in the chemical behaviour of the molecules; but the physical means by which they are recognized, their molecular electric charge, is altered sufficiently to change their motility in an electric field, and thus make them behave differently from the chemically similar amino-acids formed by the enzymic liberating processes.

If this problem is considered more in terms of physics than of chemistry, it will be seen that the application of wave mechanics to the behaviour of electrons will lead to an understanding of the function of the ultra-structure of the living intracellular system, and to an appreciation of factors hitherto thought to be insignificant.

It is suggested therefore that, under the readily observable chemical changes in heat-catalysed hydrolysis, there lie appreciable variations in the world of physical parameters. There are a number of changes in the physical nature of the molecules which may occur, but which will not affect necessarily the simple chemical behaviour of the amino-acids. They will, however, become apparent when tested by sufficiently sensitive methods, for example, that of molecular electric charge measurement.

Unfortunately, it was beyond the scope of the present research to investigate the demonstration of the differences in molecular electric charge by physical means other than high-tension electrophoresis.

However, it is thought to be more than probable from what is known of the forces involved in crystallization, that the existence of differences in molecular

electric charge on one or two sites of an amino-acid molecule would result in alteration, if only to a limited extent, of its crystalline structure. Nevertheless, I have been unable to find any reference in the literature to work on the crystalline structures of amino-acids derived from different means of liberation.

Another means by which it would apparently be possible to prove or disprove the above theory would be to measure the electron spin resonance of these differently liberated amino-acids. This excellent method, although in its pioneer stage, promises to become a most powerful tool for fundamental research, and is already finding its way into biological laboratories at the present time[20,21].

The measurement of the diamagnetic susceptibility of amino-acids[22] of different origin would also be a useful method of investigation. In 1936, Bauer and Ruskin[23] used this method with great success in establishing the differences between the diamagnetic susceptibility of proteins in living and non-living states. Work along similar lines is in progress, to interpret their results in the light of the present findings with regard to molecular electric charge and its connexion with the electrical functions of living systems, and will be described later in a separate publication.

I thank G. H. Zeal and Co., London, for their help in designing and supplying special thermometers for the observation of temperature variations along the entire length of the high-tension electrophoresis paper. This has enabled me to clear up an important point and one which has been in question for many years.

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BIO-ASSAY OF ERYTHROPOIETIN IN MICE MADE POLYCYTHÆMIC BY EXPOSURE TO AIR AT A REDUCED PRESSURE

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ERYTHROPOIETIN is characterized by its ability to induce an increase in the total red cell mass of an intact normal animal[1]. This property is seldom used for the estimation of erythropoietic activity in biological fluids from individual animals or human subjects, since it requires larger amounts of active material than are usually available. For this reason, methods of assay in common use employ test animals in which red cell formation has been suppressed[2,3] so that there is a low base-line from which to measure stimulation of erythropoiesis. Formation of new red cells is estimated by measurement of parameters which reflect maturation and division of bone marrow cells[4], hæmoglobin synthesis[5–7], or a mixture of these events. The assays in most general use are based on measurement of reticulocyte counts[8–12] or incorporation of iron-59 into circulating red cells[5–7]; the test animals are normal[10,11] or starved[3,5] or transfused-polycythæmic rats[7] or mice[9]. This article describes a system for the bio-assay of erythropoietin in mice in which endogenous red cell formation is suppressed by the polycythæmia which persists after animals have been exposed to air at a reduced pressure; incorporation of iron-59 into red cells is used as the response parameter to measure the stimulation of new red cell formation produced by the administration of exogenous erythropoietin.

The erythropoietin used in development and use of the assay was prepared from plasma of rabbits, monkeys or sheep, made anæmic by treatment with phenylhydrazine. The rabbit material was a filtrate made from plasma after heat treatment at *p*H 5·5 essentially as described by Borsook[13]; it was stored at −30° C. The monkey material was prepared from the supernatant of plasma after treatment with 15 per cent w/v sodium sulphate. The sheep material was a freeze-dried powder (Lot No. *RO*77019), purified in the Armour Laboratories and kindly provided by Dr. White; it was made up in solution so that 1 ml. contained 8·0 'cobalt units'[14]. The human erythropoietin was unfractionated plasma from an anæmic patient with paroxysmal nocturnal hæmoglobinuria. The test animals were female mice of the MRC *TO* and Schneider strains and weighed 16–24 gm. Before the test, they were maintained for 14 days in a tank with air at a pressure of half an atmosphere, and an air flow through the tank sufficiently great to keep the carbon dioxide concentration below 1 per cent. Water and food (MRC cube diet 41) were supplied

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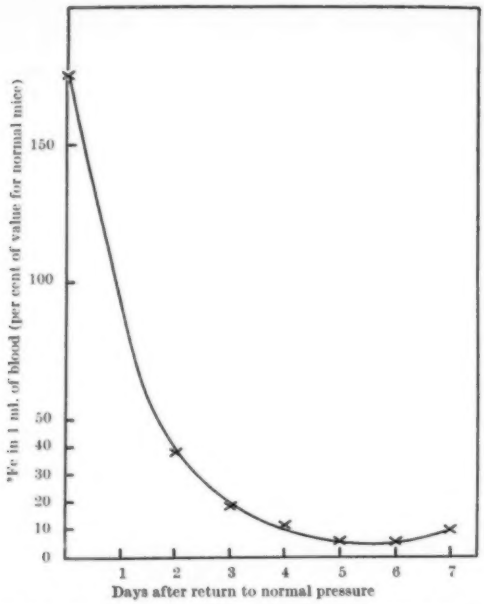

Fig. 1. Depression of red cell formation in mice returned to air at normal pressure after two weeks in air at half an atmosphere pressure
Appearance of a tracer dose of iron-59 in 1 ml. of blood of female *TO* mice (20 hr. after intravenous injection of 0·1 μc. ^{59}Fe-citrate) at various time-intervals after return of the mice to normal pressure. Results expressed as percentage of iron-59 appearing in blood of normal mice

ad lib. and the tank was opened to the atmosphere for a short period each day for routine animal care. Hæmatocrits were estimated by a micro-method. Blood volume was measured using red cells labelled with chromium-51 which, before use, were screened by passage through a normal mouse to remove cells damaged during labelling. Red cell mass was derived from hæmatocrit and blood volume. The incorporation of iron-59 into red cells was measured 20 hr. after intravenous injection of a tracer dose of 0·1 μc. iron-59 citrate; a 20-hr. time-interval was selected for this measurement, as the appearance of iron-59 in red cells was then small and less than 1 per cent of the injected iron-59 remained in the plasma.

Mice which had spent 14 days in air at half an atmosphere were studied at various times after they had been returned to normal pressure. Four or six days after the mice had been returned to normal pressure, the incorporation of iron-59 into red cells was depressed (Fig. 1), their red cell masses were increased (Fig. 2) and, in MRC *TO* mice at 4 days and 6 days, their hæmatocrits were above the normal range (Fig. 3). Blood volumes at (14 + 4) and (14 + 6) days were approximately 7·5 per cent of body-weight.

Fig. 2. Total red cell volume and body-weight of female MRC *TO* mice exposed to air at half an atmosphere pressure for 14 days and then returned to normal pressure for 6 days (series '14 + 6')
○, Normal; ●, '14 + 6'

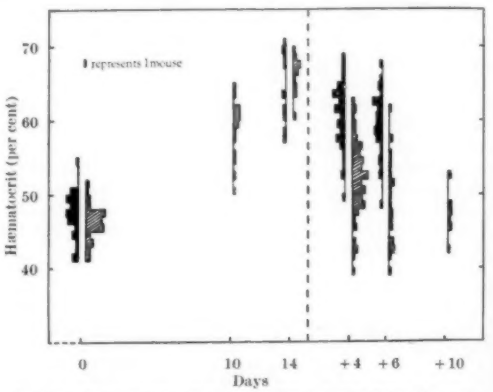

Fig. 3. Hæmatocrits of female MRC *TO* and Schneider mice exposed to air at half an atmosphere pressure for 14 days and then returned to normal pressure. Frequency distribution of hæmatocrits are shown as histograms
Black, *TO*; shaded, Schneider

On the basis of these results, the response to erythropoietin was investigated in mice which had spent 14 days in air at half an atmosphere pressure and which were killed 4 or 6 days after their return to normal pressure. These series are referred to as '14 + 4' and '14 + 6', respectively. The routine of the test is essentially similar to that used by other workers and is shown in Table 1. The response was taken as the percentage of the administered iron-59 present in the blood 20 hr. after intravenous injection of the iron-59; the blood volume was calculated as 7·5 per cent of body-weight. The log dose–response relationships obtained with small groups of mice treated with rabbit or sheep erythropoietin are shown in Fig. 4. From these graphs it may be seen that both the MRC *TO* and the Schneider mice are satisfactory test animals for the estimation of erythropoietin. *TO* mice, series 14 + 4, have been selected for our routine assay of erythropoietin. With rabbit erythropoietin, the log dose–response regression line did not differ significantly from linearity over a thirty-fold range of doses; its slope (b) was 16·03 and the index of precision (λ) was 0·35. Sheep erythropoietin tested in this system also gave a linear regression with slope 20·73 and index of precision (λ) 0·22. Over a longer range of doses, the log dose–response relationship was sigmoid in form. In this series the range of hæmatocrits of test animals was above the normal. Thus measurement of hæmatocrit was used to assess whether a test extract had

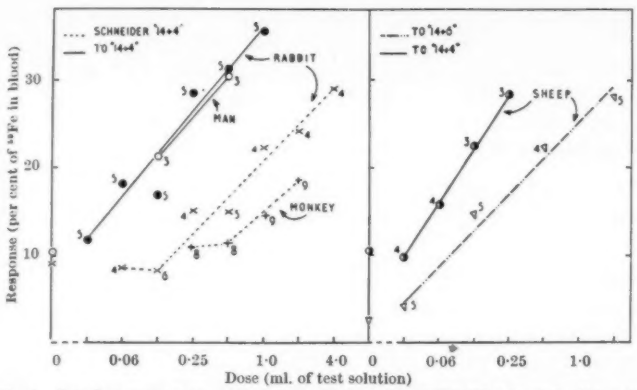

Fig. 4. Log dose–response regression lines obtained with MRC *TO* mice series '14 + 4' and '14 + 6' and with Schneider mice series '14 + 4' treated with preparations of erythropoietin from plasma of rabbit, sheep, monkey and man. Figures on the graph show numbers of animals used

caused hæmolysis, so that affected animals in which response might be increased by secretion of endogenous erythropoietin might be excluded from the test (cf. Winkert and Gordon[15]). In routine assays, results from mice with hæmatocrits below 50 per cent were discarded.

TO mice killed on day (14 + 6) may be advantageous for certain assays. These animals gave a base-line which was lower and more consistent than that obtained with the '14 + 4' series. They were sensitive to as little as 0·25 'cobalt units'[16] of erythropoietin and responded to this with a significant increase above the base-line incorporation of iron-59. The hæmatocrits of *TO* mice, series '14 + 6', overlapped little with the normal range and were also used to show whether treatment had caused hæmolysis. The Schneider mice, series '14 + 4', were not selected for routine assays because they were less sensitive to small doses of erythropoietin than the *TO* mice, and their range of hæmatocrits overlapped with the range for normal Schneider mice.

Data obtained with human erythropoietin administered to *TO* mice, series '14 + 4', and with monkey erythropoietin administered to Schneider mice, series '14 + 4' are also included in Fig. 4. These show that the system responds to erythropoietins prepared in various ways from four species, namely, rabbit, sheep, man and monkey; moreover, the slopes of the regression lines obtained with rabbit, sheep and human erythropoietin did not differ significantly ($P = 0{\cdot}35$).

Table 1. Routine Test Procedure for use of Mice made Polycythæmic by Exposure to Hypoxia

	Day of test	
	Series '14 + 4'	Series '14 + 6'
Mice maintained in air at half atmosphere pressure	0–14	0–14
Mice returned to normal pressure	14	14
First injection of test material (68 hr. before killing)	15	17
Second injection of test material (44 hr. before killing) (Each injection made up to 1 ml. with saline and given intraperitoneally)	16	18
^{59}Fe-citrate, 0·1 μc. in 0·2 ml. saline, injected intravenously (20 hr. before killing)	17	19
Weigh, bleed and kill mice (avertin anæsthetic)	18	20
Measure hæmatocrit and count ^{59}Fe present in sample of blood using a well-type scintillation counter		

This method has certain advantages over the methods in common use for estimation of erythropoietic activity. The labour and cost of transfusion, and the possibility of non-specific stimulation in a starved animal is avoided by the suppression of endogenous erythropoiesis with a polycythæmia of each animal's own red cells. Our test animal is a more physiological preparation than a starved or transfused animal. The mice are easily prepared in large numbers and the assay may thus be planned on a statistical basis. The system is at least as sensitive as other *in vivo* methods for the estimation of erythropoietin[17].

Garcia[6] has shown that a preparation of human urinary erythropoietin which stimulates incorporation of iron-59 into red cells in the starved rat, will also increase the total red cell mass in rats. Keighley *et al.*[18] have shown that one preparation of rabbit plasma erythropoietin induced an increase in the total red cell volume, hæmoglobin concentration and hæmatocrit of normal rats; it stimulated incorporation of iron-59 into red cells of normal, starved and hypophysectomized rats and caused a reticulocytosis in normal rats and polycythæmic-transfused mice. These test systems reflect various aspects of erythropoiesis. The incorporation of iron-59 into red cells measures chiefly hæmoglobin synthesis and only indirectly reflects division of erythropoietic cells. Reticulocytosis measures chiefly maturation of normoblasts, but it may be produced by release of reticulocytes. At the present time it is not known if these and other aspects of erythropoiesis are all controlled by one and the same 'erythropoietin' molecule. It is therefore essential that estimates of the relative erythropoietic activities of various preparations should be correlated by simultaneous studies using different assay systems.

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TRYPTAMINE RECEPTORS IN THE CENTRAL NERVOUS SYSTEM

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THE actions and role of 5-hydroxytryptamine (5-HT) in the central nervous system are of great interest. Since injected 5-HT does not readily enter the central nervous system, Vane[1] suggested studying the central actions of related compounds such as α- or *N*-alkyl-tryptamines and their 5-methoxy derivatives, which are able to pass the blood-brain barrier and resist attack by monoamine oxidase (MAO). We describe here some effects of tryptamine, *dl*-α-methyltryptamine (MT), *dl*-α-ethyltryptamine (ET), *dl*-5-methoxy-α-methyltryptamine (MeOMT) and *N*,*N*-dimethyltryptamine (DMT) on the behaviour of animals and on their responses to other centrally acting drugs, on central electrical activity and on spinal reflexes. The 5-HT-like actions of these compounds on isolated smooth muscle have already been described[1,2].

Spontaneous Locomotion in Mice

Locomotion was measured as the number of breaks of an infra-red beam in modified Dews[3] activity boxes. Administered intraperitoneally to normal mice, MT, ET and, less strongly, MeOMT stimulated spontaneous locomotion, while DMT was inactive (Table 1). Tryptamine, 5-HT and iproniazid depressed locomotion, confirming the results of Brown[4]. Intravenously, however, tryptamine produced a brief episode of excitement followed by a prolonged period of depression.

Table 1. Potencies of Tryptamines and Other Compounds in Mice

Compound	Effective dose (mgm./kgm. i.p.) producing stimulation (*S*) or depression (*D*) of locomotion *	Effective dose (mgm./kgm. i.p.) producing reversal of reserpine ptosis‡
Tryptamine	50 (*D*)	190
α-Methyltryptamine	12·5 (*S*)	9·4
α-Ethyltryptamine	12·5 (*S*)	28·3
5-Methoxy-α-methyltryptamine	50 (*S*)	> 60 §
N,*N*-Dimethyltryptamine	No effect at 50	42·5
5-Hydroxytryptamine	50 (*D*)	> 300 ‖
Iproniazid	> 400 (*D*)†	No effect at 500
β-Phenylisopropylhydrazine	50 (*S*)	33
Amphetamine	2 (*S*)	5·8

* Locomotion was measured as the number of breaks of an infra-red beam in modified Dews (ref. 3) activity boxes. The effective dose is that which increased or decreased geometric mean activity count by a factor of 3 compared with that of control mice ($P < 0.05$), when given within the dose-range 12·5, 25, 50, 100, 200 and 400 mgm./kgm. or in the case of amphetamine 1, 2 and 4 mgm./kgm.

† At this dose locomotion was depressed ($P < 0.1$).

‡ Ptosis was induced by intraperitoneal injection of 4·0 mgm./kgm. reserpine 18 hr. before the test. The effective dose is that which in a group of 10 mice gives a score for reversal of ptosis of 10 out of a possible maximum of 20 on the scale 0 = full ptosis; 2 = eyes fully open; 1 = any intermediate state between 0 and 2.

§ At this dose a maximum score of 8/20 was obtained.

‖ At this dose a maximum score of 9/20 was obtained.

In another series of experiments, mice were given a larger dose of β-phenylisopropylhydrazine (PIH) than that necessary to inhibit brain MAO completely[5,6]. 18 hr. later, we treated them with MT, ET or iproniazid. Table 2 shows that MT and ET powerfully stimulated locomotion in normal mice and in mice after inhibition of brain MAO.

Table 2. Effects of α-Methyltryptamine (MT), α-Ethyltryptamine (ET) and Iproniazid on Locomotory Activities of Normal Mice and Mice treated with MAO Inhibitor (β-Phenylisopropylhydrazine (PIH) 40 mgm./kgm. intraperitoneally 18 hr. previously)

Exp. No.	Treatment dose/kgm. i.p.	Mean activity count * and significance of difference	
		Without PIH	After PIH
1	Saline, 10 ml. MT, 20 mgm.	279 1,652 } $P < 0.001$	236 2,771 } $P < 0.001$
2	Saline, 10 ml. ET, 20 mgm.	102 1,355 } $P < 0.001$	169 3,751 } $P < 0.001$
3	Saline, 10 ml. Iproniazid, 100 mgm.	108 100 } $P > 0.75$	236 145 } $P > 0.10$

* Each value is the geometric mean of counts obtained over 4 hr. from 6 activity boxes, each containing 3 mice.

Anti-Reserpine Activity in Mice

Anti-reserpine activity was measured by the extent of reversal of ptosis produced by injecting reserpine 4·0 mgm./kgm. intraperitoneally 18 hr. previously. Table 1 shows that the tryptamines were active in this test, MT being the most and 5-HT the least potent. Amphetamine and PIH were also potent; but iproniazid ineffective. However, when given some hours before reserpine, iproniazid prevents ptosis[7].

Experiments on Unrestrained Conscious Cats

Intravenous tryptamine caused convulsions, tonic extension of the limbs, unsheathing of claws, hissing and snarling, piloerection, salivation, mydriasis and retraction of the eyelids: all these effects have already been described by Laidlaw[8]. The animals behaved as though angry or afraid. These reactions lasted for about 5 min., after which the animals were subdued for some hours. However, if MAO had been inhibited by injection of PIH (10 mgm./kgm. intraperitoneally 18 hr. beforehand), the phase of excitement following tryptamine lasted several hours. In untreated cats, MT or ET (5 mgm./kgm. intravenously) produced excitement lasting 4–5 hr.

Intraperitoneally, MT, ET or DMT (0·5–2·0 mgm./kgm.) caused recurrent extensor and flexor spasms of the limbs, ataxia, apparent reluctance to move and bewilderment. These effects, which lasted

for more than 6 hr., were reduced or abolished within 2 hr. by intraperitoneal injection of 2 mgm. bromlysergic acid (BOL).

In cats with implanted cortical electrodes, MT, ET, MeOMT or DMT (2 mgm./kgm. intraperitoneally) did not produce electrocortical alerting. Within 15 min., however, bursts of 6–10 c./sec. high-voltage (250–400 μV.) electrocortical activity appeared and these were maintained unchanged for more than 6 hr.

Cat Encéphale Isolé Preparations

MT (0·5–2·0 mgm./kgm. intravenously) produced immediate and prolonged behavioural arousal. There was also very brief electrocortical alerting, which was replaced in 30 min. by bursts of high-voltage electrical activity, similar to that described above in conscious cats, or by high-voltage 2–4 c./sec. activity. These changes were found in all the cortical areas except the hippocampus. MT modified the normal reciprocal relationship which exists between electrocortical activity in the hippocampus and in other areas. The bursts at 2–4 or 6–10 c./sec. seen in the cortex could also be recorded from deep structures, such as the thalamic nuclei and the reticular formation in the brain stem. Subsequent intravenous injection of 2 mgm. BOL abolished or reduced the electrical patterns attributable to MT gradually over 1–2 hr.

In addition to changing spontaneous electrical activity, MT also affected evoked potentials. Thus the threshold for electrocortical arousal on stimulating the brain-stem reticular formation was raised from 0·4 to 1·2 V. and that on stimulation of the thalamic nuclei from 2·5 to 4·0 V. Similarly, the thresholds for eliciting recruiting and augmenting responses by low-frequency stimulation of the appropriate thalamic nuclei were raised as were the thresholds for arousal with tactile and auditory stimuli.

Spinal Reflexes

These were studied in spinal or chloralosed cats. In a polysynaptic reflex (twitch of the tibialis anterior muscle evoked by stimulation of the central end of the cut posterior tibial nerve), increase of both basal tension and peak twitch tensions with fused or partially fused tetani followed intravenous injection of tryptamine, MT, ET, DMT or MeOMT (0·5 mgm./kgm.). The facilitating effect of tryptamine was brief and larger doses abolished the response. However, with tryptamine after MAO inhibition or with MT alone, a tetanic response was followed by protracted augmentation of the twitch and a single sensory nerve stimulus produced repetitive muscle contractions. These compounds similarly facilitated a monosynaptic reflex (knee jerk).

Several observations showed that the facilitating effects of the tryptamines originated in the spinal cord: (1) these substances increased the spike potential recorded from the ventral root after stimulation of the corresponding dorsal root; (2) they were more potent when injected into a spinal artery; (3) they did not enhance responses of muscle to direct stimulation of the motor nerve or of the muscle itself; and (4) pressor effects of comparable size to those produced by tryptamines, but elicited by tyramine, adrenaline or noradrenaline, did not increase the reflexes.

Reduction of the spike potential after the initial increase sometimes followed injection of tryptamine. 5-HT (1 mgm./kgm. intravenously) enhanced both reflexes, but after a much longer latency, possibly because 5-HT does not easily cross cell barriers[1]. BOL (2 mgm. intravenously) and another specific antagonist of 5-HT—1-methyl-D-lysergic acid butanolamide[9] (UML-491)—at the same dose, reduced or abolished these reflexes, and also the facilitatory actions of the tryptamines.

Depression of both reflexes by acute or chronic reserpine or acute chlorpromazine treatment was reversed by intravenous injection of any of the compounds, MeOMT being the most potent.

Conclusions

In all the effects we have studied, tryptamine differed from MT and ET only in depressing spontaneous locomotion in mice, although for a brief initial period it caused excitement. Greig *et al.*[10,11] have claimed that MT and ET owe their central stimulatory action to inhibition of MAO, causing an accumulation of catechol amines or HT in the brain. However, two reasons show that MT and ET do not only or even mainly act in this way: (1) their stimulatory effect on locomotion was undiminished by prior inhibition of MAO (Table 2); and (2) they take effect rapidly. Another explanation of the action of substituted tryptamines has been advanced[12]—that they increase the availability of free HT by preventing its storage. However, this explanation seems unlikely to be important, since the substituted tryptamines act after reserpine, which has already inhibited storage of HT. These considerations suggest that the substituted tryptamines act directly on receptors in the central nervous system. Gaddum[13] has argued that tryptamine and 5-HT share common receptors in the gut, and Tedeschi, Tedeschi and Fellows[14] have advanced a similar hypothesis for the central nervous system. That substituted tryptamines as well as tryptamine and HT are antagonized by BOL and UML-491 suggests that they all act on the same receptors—the tryptamine receptors. That electrical responses to substituted tryptamines can be recorded from cortex, thalamus, brain stem and spinal cord suggests that tryptamine receptors are widely scattered throughout the central nervous system. In spinal reflexes in particular, the high potency of tryptamines and their antagonism by specific antagonists of 5-HT suggest that tryptamines naturally present may have a physiological function.

The substituted tryptamines were prepared in the Department of Chemical Research, Parke, Davis and Co., Hounslow. We wish to thank Dr. R. E. Bowman for helpful discussions, Dr. B. T. Warner for statistical advice and Mr. R. W. Pickering for experimental assistance. We also wish to thank Ciba Laboratories, Ltd., for supplies of reserpine and Sandoz Products, Ltd., for UML-491.

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INHERITANCE OF SUSCEPTIBILITY TO INDUCED PULMONARY TUMOURS IN MICE

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THE inheritance of cancer susceptibility is not amenable to study by the methods of Mendelian genetics, because many genes are involved which cannot be recognized individually, and any measure of the degree of susceptibility is subject also to non-genetic variation[1]. The methods of quantitative genetics must, therefore, be applied, and this article describes briefly the results of an investigation by these methods. Pulmonary tumours induced by urethane were chosen for study, because the manifestation of this form of cancer can be measured quantitatively, by the number of tumours present. All mice were given the same treatment: 0·1 ml. of 10 per cent urethane in distilled water injected intraperitoneally at 3 weeks of age, followed by a second injection of 0·28 ml. at 9 weeks. The mice were dissected at 23 weeks of age and the tumour nodules visible to the unaided eye on the surface of the lungs were counted. The number of tumours counted were transformed to square roots for the purpose of computation.

The object of the work was to determine the relative importance of heredity and environment as causes of the differences of tumour number between the individuals of a genetically heterogeneous, or random-bred, strain. The observed variance, that is, the phenotypic variance (V_P) among the individuals of a genetically heterogeneous strain is the sum of two principal components, the genetic variance (V_G) and the non-genetic, generally termed the environmental, variance (V_E); so that:

$$V_P = V_G + V_E$$

Then the ratio of the genetic to the total variance, V_G/V_P, measures the relative importance of genetic differences between individuals in determining the differences of tumour number between them. This partitioning of the variance can be made if a group of genetically homogeneous individuals is available, such as the F_1 of a cross between highly inbred lines. The variation among these, which contains no genetic component, provides a measure of the non-genetic variation; and, if this can be assumed to represent the non-genetic variation in the non-inbred strain, an estimate of the genetic variance can be obtained by subtraction. The genetic variance can be subdivided into an additive component (V_A), which is the variation chiefly responsible for the resemblance between relatives, and the remainder, or non-additive component (V_{NA}), which arises from dominance and other genetic complexities. The additive genetic variance can be estimated from the resemblance between relatives in a genetically heterogeneous strain. Thus the measurement of the variances of a genetically heterogeneous strain and of a genetically uniform group, together with the measurement of the resemblance between relatives in the heterogeneous strain, leads to a partitioning of the phenotypic variance of the heterogeneous strain as follows (for a more complete explanation, see ref. 2):

$$\underset{\text{(phenotypic)}}{V_P} = \underbrace{V_A + V_{NA}}_{\text{(genetic)}} + \underset{\text{(non-genetic)}}{V_E}$$

The ratio of the additive genetic variance to the total (V_A/V_P), known as the heritability, measures the extent to which differences of tumour number are transmitted from parents to offspring. Only in the absence of genetic complexities giving rise to non-additive variance does it measure the degree of genetic determination, or the relative importance of genetic differences in determining the phenotypic differences between individuals.

The genetically heterogeneous strain used in this work, known in the laboratory as *JC*, originated from crosses between several non-inbred strains and had been maintained for some thirty generations by random mating. The genetically uniform mice, from which the non-genetic variance was estimated, consisted of thirty F_1's made from six highly inbred strains crossed in all combinations, and the inbreds themselves. The variances of the inbreds did not differ from those of the F_1's, so there was no reason to exclude them. Counts were made on 723 F_1 and inbred individuals. The mean tumour number ranged from 0·9 to 25·4, with an overall mean of 11·7. (In what follows the F_1's and inbreds together will be referred to as the uniform groups.)

The results are shown in Table 1. The transformation to square roots did not fully succeed in rendering the variance independent of the mean, and the variance in the uniform groups tended to be higher in those with higher means. Since most of the groups had higher means than the heterogeneous strain, it was therefore necessary to adjust the estimate of the uniform group variance to allow for this difference. This was done from the regression of the standard deviation on the mean, which led to an estimate of the expected variance of uniform groups having the same mean tumour number as the heterogeneous strain. The unadjusted estimate of the mean variance in the uniform groups was 0·527, and the adjusted estimate was 0·325. The comparison of the variances of the heterogeneous strain and the uniform groups adjusted in this way leads to the conclusion that 58 per cent of the variation in tumour number in the heterogeneous strain

Table 1

Total phenotypic variance of random bred strains (V_P)	0·771 ± 0·042
Phenotypic variance of uniform groups (adjusted) (V_E)	0·325
Total genetic variance of random-bred strain ($V_G = V_P - V_E$)	0·445
Degree of genetic determination (V_G/V_P) = 0·445/0·771 =	58 per cent
Regression of offspring on mid-parent	0·323 ± 0·051
Correlation of full sibs	0·218 ± 0·044

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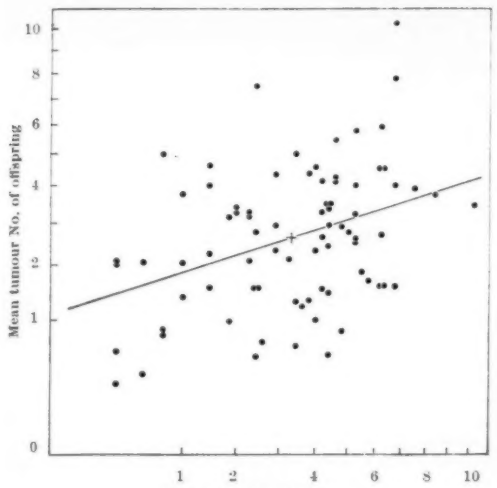

Fig. 1

was genetic in origin. This measures the relative importance of heredity as a cause of differences in tumour number.

The additive genetic variance in the heterogeneous strain was estimated from the regression of offspring on the mean of their two parents. Counts were made on 518 offspring in 79 families. The mean tumour number was 3·4. The correlation of full sibs (litter-mates) was also estimated. As a measure of the additive genetic variance this is liable to upward bias from non-additive genetic variance and from environmental causes of variation common to litter-mates, such as might arise from maternal effects or from the experimental procedure. The scatter diagram shown in Fig. 1 illustrates the resemblance between offspring and parents. Each point represents the mean tumour number among the offspring of one pair of parents plotted against the mean tumour number of the parents. The straight line is the computed regression of offspring on mid-parent (that is to say, the mean of the two parents), and the slope of this line measures the degree of resemblance between offspring and parents, which estimates the heritability (V_A/V_P). This was $32 \pm 5{\cdot}1$ per cent. Thus, the heritability differs materially from the ratio V_G/V_P, which was 58 per cent. This indicates the presence of non-additive genetic variance arising from dominance and other genetic complexities in the action of the genes controlling tumour number. If there were no non-additive genetic variance and no maternal effects the full-sib correlation would estimate half the heritability. Doubling the observed correlation yields an estimate of $44 \pm 9{\cdot}2$ per cent. This is in excess of the estimate from the offspring–parent regression, which is to be expected when non-additive variance is present.

These analyses show that about 58 per cent of the variation in tumour number between the mice of the strain studied was caused by genetic differences. The remaining 42 per cent, which is non-genetic in origin, can be subdivided by consideration of the fact that the number of tumours counted does not necessarily indicate precisely the true susceptibility of the individual. A number of individuals of exactly the same susceptibility would be expected to vary in the number of tumours present, because the occurrence of a tumour can be regarded as a chance event with a certain probability characteristic of the susceptibility of the individual. The variance of tumour number will, therefore, have a component of variance attributable to chance in addition to the variance arising from individual differences of susceptibility. There is evidence[3] that the chance variation follows a Poisson distribution; the variance attributable to chance will then be equal to the mean tumour number[4], when the mean and variance are computed from the untransformed counts. The mean of the (untransformed) tumour numbers in the random-bred strain was 3·4 and the variance was 8·5. The variance attributable to chance (that is, 3·4) therefore represents 40 per cent of the total variance of tumour number. This leaves only 2 per cent of the variance of tumour number to be attributed to non-genetic differences of susceptibility together with differences due to experimental error, such as might have arisen from variation in the dosage of urethane administered to different individuals. It is not altogether credible that non-genetic differences of susceptibility, together with experimental error, account for as little as 2 per cent of the variation. The chief source of possible error in this estimate lies in the adjustment of the variances of the uniform groups, and it is not a simple matter to attach a standard error to the adjusted estimate. A second source of possible error lies in the amount of variance to be attributed to chance. Until these sources of error have been excluded, or confirmatory evidence obtained from other experiments, only a qualitative conclusion can be accepted, namely, that environmental sources of variation are relatively unimportant, and an individual's genetic constitution is the major determinant of its true susceptibility to urethane as a carcinogenic agent.

The results of this work are summarized in Table 2, which shows the relative importance of heredity, environment and chance as causes of the differences between individuals in the number of tumours developed. The figures are subject to some possible error, but the following conclusions can be drawn. More than half the variation of tumour number among the mice of the strain investigated results from genetic differences between the individuals; but, as a result of genetic complexities, only about one-third of the variation is 'inherited' in the sense of being transmitted from parents to offspring. A major part of the non-genetic variation of tumour number is attributable to chance, and environmental differences between individual mice are relatively unimportant as determinants of an individual's susceptibility to the carcinogenic action of urethane.

Table 2

	Total (per cent)
Genetic variance of susceptibility	
Additive	32
Non-additive	26
Total	58
Non-genetic variance of susceptibility	2
Chance variance of tumour number	40
Total variance of tumour number	100

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A MORPHOGENETIC EFFECT OF BETA-MERCAPTOETHANOL

Distribution of Water in the Embryo

CELLULAR mechanisms responsible for the uptake and distribution of water in the embryo play an important part in normal development[1]. Prof. Brachet's reports[2] on the effects of β-mercaptoethanol on the normal development of urodele embryos, which he attributed to its effect on the fibrous proteins responsible for morphogenetic movements, were therefore of particular interest, because two of the abnormalities he described—the collapse of the blastocœl and the failure of the neural tube to close—clearly resulted in an abnormal distribution of water in the embryo.

This prompted me to carry out a series of quantitative experiments to measure the effect of this reagent on the uptake and distribution of water during the formation of the blastula and gastrual of *Xenopus leavis* (Daud.). The results were surprising, for they showed that under the conditions of my experiments, *M*/500 mercaptoethanol had little or no effect on the morphogenetic movements themselves, but it did have a pronounced effect on the uptake and, particularly, the distribution of water in the embryo.

Having first established that the reagent had no effect on the reduced weight of the embryos by weighing single embryos on a Cartesian diver balance before and after treatment for 3 hr. with *M*/100 mercaptoethanol, it was assumed that the density of the embryos could change only by taking up or losing water. (It had previously been established that the reduced weight of normal embryos remained constant until the early tail-bud stage.) Thus, it was possible to follow the effect of the reagent on the water content of the embryo by measuring the density of treated and control embryos in a density gradient of colloidal thorium oxide in water[3]: and its effect on the volume of the cells themselves could be determined by repeating the process on open embryos from which the contents of the intercellular spaces had been removed. The results of one such experiment are shown in Table 1, and the corresponding volume changes, calculated from the mean densities and the mean reduced weight of the experimental embryos, are shown in the histogram (Fig. 1).

It will be seen that at 10 hr. there is very little difference between control embryos and embryos

Table 1

Control embryos in buffered tap water *p*H 7·4 at 20° C. Treated embryos in buffered tap water *p*H 7·4 at 20° C. + *M*/500 β-mercaptoethanol

	Duration of treatment Age (hr.)		No. of embryos	Mean density (20° C.) at end of treatment	
	In	Out		Intact	Open
				gm./ml.	
Control	—	—	5	1·0770	1·0887
Treated	2	10	5	1·0780	1·0887
Control	—	—	6	1·0620	1·0840
Treated	2	22	5	1·0732	1·0812

laid at the same time which had been in a solution of *M*/500 mercaptoethanol from the four-cell stage onwards. The volume of the cells in both cases is the same. At 22 hr., however, there is a marked difference. The density of the treated embryos is very much greater than that of the controls, whereas the density of their cells is slightly less. This means that the volume of the intercellular cavities of the treated embryos is very much less than that of the controls (Fig. 1). The treated embryos differ from the controls in one other important respect—they lie upside down in the vitelline membrane, due to the presence of a liquid-filled cavity on their ventral surface. The presence of this cavity became more pronounced if treated embryos were removed from the reagent at this stage and transferred to tap water (Fig. 2).

Serial sections and time-lapse films clearly showed that this cavity was the cavity of the blastocœl which, having formed normally, failed to empty during gastrulation. The morphogenetic movements involved in the formation of the archenteron and its associated structures did not appear to be affected by the treatment, and all the structures normally found in transverse sections of normal embryos could be identified. The only obvious abnormality was that the archenteron cavity was empty. At a later stage the treated embryos also differed from the controls, as Brachet reported, in having open neural tubes. It should be mentioned in passing that nuclear counts from macerated embryos showed that treated and control embryos have the same number of nuclei.

It seems reasonable to conclude, therefore, that the primary effect of the mercaptoethanol is not, as Brachet has suggested, on the mechanisms responsible for the morphogenetic movements themselves, but on the mechanisms responsible for controlling the

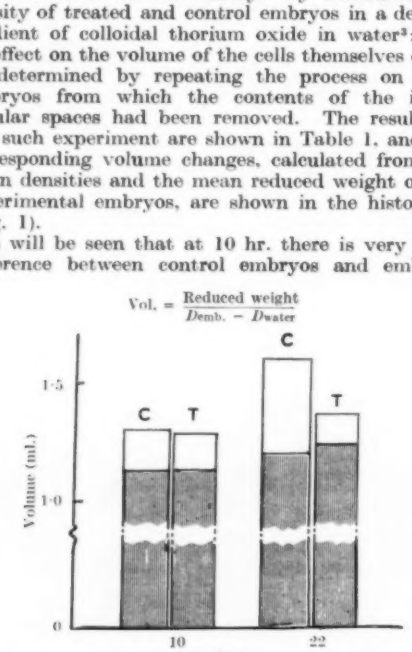

Fig. 1. Histogram showing effect of β-mercaptoethanol on the volume of *Xenopus* embryos. Shaded areas, vol. of cells; unshaded, vol. of intercellular cavities

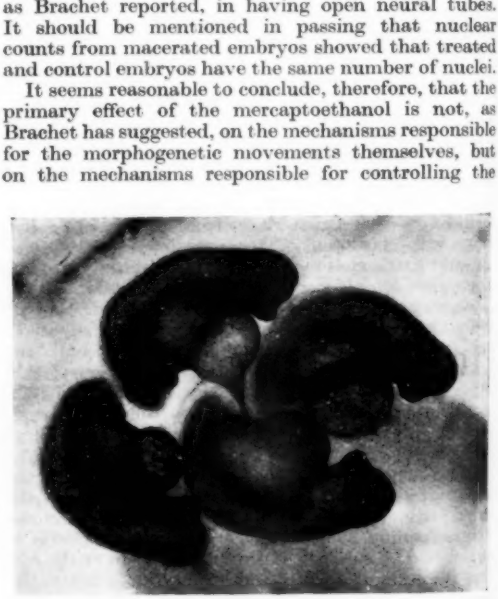

Fig. 2. *Xenopus* embryos showing persistent blastocœl cavities after 15 hr. in tap water following treatment for 20 hr. with *M*/500 mercaptoethanol at 20° C.

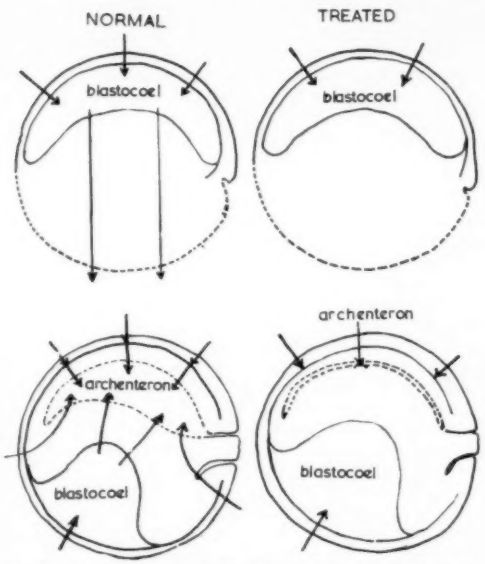

Fig. 3. Diagram to illustrate the effect of the net water fluxes in normal and treated *Xenopus* embryos

distribution of water in the embryo. This in turn may be the result of its effect on the bonding of specific proteins, for as I have shown[1], the distribution of water in the embryo is probably due to the difference in polarity of cellular mechanisms located in the cell membranes, and not to simple osmotic and hydrostatic gradients.

Thus, in the normal embryo, these mechanisms are thought to be polarized in such a way that there is a net influx of water through the cells of the animal pole and a net efflux through the vegetative pole cells, the difference between the two fluxes being responsible for the accumulation of water in the blastocœl, and after invagination for the transfer of water into the archenteron. The failure of treated embryos to transfer water from the blastocœl into the archenteron may therefore be explained by supposing that the reagent reduces the endodermal efflux to zero, leaving a small ectodermal influx to account for the formation of the blastocœl which, as the experiments show, is unaffected (Fig. 3).

This work is being reported in more detail elsewhere[4].

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Distribution of Water in the Chick Blastoderm

IT has been shown experimentally that the effect of β-mercaptoethanol on the morphogenesis of the anuran *Xenopus leavis* is largely due to an abnormal uptake and distribution of water in the treated embryos. This was explained by suggesting that the reagent affects the mechanisms responsible for controlling the water content of the embryonic cells in such a way that the normal net efflux of water through the endoderm cells is abolished, so preventing the transfer of water into the archenteron, leaving a small residual ectodermal influx to account for the formation of the blastocœl, which is unaffected[1].

The complexity of the geometrical relations between ectoderm and endoderm in the amphibian embryo makes it difficult to test this hypothesis. In the early chick embryo, on the other hand, the problem is greatly simplified because the ectoderm and endoderm are merely parallel sheets of cells. Moreover, their polarity has been demonstrated experimentally by New, who has shown that water is transferred through the chick blastoderm from the ectodermal to the endodermal surface[2]. Thus not only is the polarity the same as that postulated for the *Xenopus* embryo, but there must also be a similar difference in the magnitude of the fluxes to account for the small accumulation of liquid between the layers which occurs during the development of the normal blastoderm.

Accordingly, one of us (A. J.) has carried out a series of experiments to test the effect of β-mercaptoethanol on early chick embryos.

Chick embryos were explanted from eggs at the definitive streak stage (Hamburger and Hamilton stage 4)[3] and cultured *in vitro* using the technique described by New[3]. The β-mercaptoethanol was diluted with liquid egg albumen containing 10 per cent sterile saline solution (0·9 per cent) to give a final concentration of 4 or 5 m*M*. and 0·5 ml. of this solution was spread around the plastic ring on which the embryos were cultivated; in control experiments a solution of liquid egg albumen containing 10 per cent saline was applied in the same way. After treatment, the embryos were incubated for another 20–24 hr., that is, until the controls had reached stages 10–12 (Fig. 1). Some embryos were also treated *in ovo* at stage 4 by injecting 0·25 ml. of 400 m*M* solution of the reagent. These embryos were then allowed to develop for another 20–24 hr. before being explanted.

Macroscopic examination of treated embryos showed abnormal swelling of the blastoderm on either side of the mid-line and enlargement of the proamnion and the head. The neural tube in all cases failed to close, often along its entire length (Figs. 2 and 3).

In transverse sections it was seen that the swelling of the blastoderm was the result of abnormally large spaces between the ectoderm and mesoderm, and between mesoderm and endoderm. This was especially noticeable in the region of the posterior somites and farther towards the caudal end of the embryo (Figs. 4 and 5). The extra-embryonic cœlom was also widened along the whole length of the embryos. In the cephalic region, the mesenchyme spaces were enlarged, and the mesenchyme cells were dispersed more than in the normal embryo. The increase in size of the head in treated embryos was in part the result of a considerable dilation of the main blood vessels (ventral and dorsal aortic roots) accompanied by a dilation of the heart ventricle.

The neural plate of treated embryos, especially where it failed to close along its entire length, sometimes showed necrotic changes in the median part of the neural groove, or scattered over the whole tissue. The notochord was sometimes poorly formed and not clearly detached from the surrounding mesenchyme cells, and finally, in some embryos, well-defined blisters were found in both the ectoderm and endoderm.

Fig. 1. Control chick embryo at stage 10 (× 15)
Fig. 2. Chick embryo after treatment with $M/250$ solution of β-mercaptoethanol. Note the open neural tube and widened omphalomesenteric veins (× 15)
Fig. 3. Chick embryos treated with $M/200$ solution of β-mercaptoethanol. Note the enlarged head, and open neural tube (× 20)
Fig. 4. Transverse section through a control embryo (stage 10) at the level of the last somite (× 40)
Fig. 5. Transverse section through an experimental embryo at the same level after treatment with $M/200$ β-mercaptoethanol (× 40)

These results show that in the chick, as in *Xenopus*, β-mercaptoethanol causes an abnormal distribution of water in the embryo: a distribution, which is in accord with the hypothesis outlined earlier. Thus if mercaptoethanol reduces the endodermal efflux in the chick as it does in *Xenopus*, the residual ectodermal influx would account for the abnormal accumulation of water between the two layers that has been observed.

The effect of the reagent on the volume of the cœlom is less easily explained. However, the fact that the myocœl when it forms is not abnormally large, although the extra embryonic cœlom is always greatly distended, suggests that the mesodermal cells are normally polarized centripetally with respect to the net transfer of water; but because the lateral plate mesoderm is so closely associated with the ectoderm and endoderm, the layers of cells which result—the somatopleur and splanchnopleur—have the same polarity as the ectoderm and endoderm respectively and are affected in the same way.

A further interesting feature of these experiments is the appearance of the neural plate in transverse sections of treated embryos; this suggests that its failure to close may simply be a mechanical consequence of the increased tension in the ectoderm caused by the abnormal accumulation of water. Attempts to test this hypothesis by puncturing the ectoderm of embryos that had been treated *in ovo* (see earlier) were inconclusive. The neural plate did not close up immediately the tension in the ectoderm had been relieved, although there was a limited closure after 2 hr. The embryos in these experiments were of course relatively old, and it is possible that permanent damage had occurred in the neural tissues. Moreover, the reagent may affect the differential water uptake of the cells on the upper and lower surfaces of the neural plate itself; a differential which must exist in the normal embryo to account for the changes in cell volume which occur during invagination.

In view of these results, it is suggested that the effects of other specific morphogenetic inhibitors, particularly those which tend to alter the S—S S—H equilibrium of the cells, may be the result at least in part of their effect on the distribution of water in the embryo and the mechanisms by which it is controlled.

A detailed account of this work will be published elsewhere.

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IONIC CURRENTS IN MEMBRANE OF ACTIVE MUSCLE FIBRE

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A NERVE or muscle fibre immersed in a large volume of fluid and carrying a propagated action potential at velocity s, will obey the following relationship, usually termed the cable equation:

$$(a/2Rs^2)\mathrm{d}^2V/\mathrm{d}t^2 = C\ \mathrm{d}V/\mathrm{d}t + I_i \qquad (1)$$

where a is the radius of the fibre, R is the resistivity of the cytoplasm, V is the membrane voltage, t is time, C is the membrane capacitance, and I_i is the membrane ionic current density. As a rule, this equation cannot be integrated since I_i is an unknown function of voltage and time. However, if the first and second time-derivatives of the action potential can be determined as functions of time, appropriate substitution into (1) will lead to a numerical solution for the membrane ionic currents associated with the action potential.

During investigation in this laboratory of the action potential of frog sartorius fibre, it was realized that much of the needed information lies in a record of the action potential in phase-plane co-ordinates, as in Fig. 1. Trans-membrane voltage (V) was recorded on the horizontal axis of the oscilloscope, while dV/dt (obtained electronically) was recorded simultaneously on the vertical axis. The entire spike-potential record develops clockwise from the initial spot at the left (resting potential − 92 mV.). The farthest horizontal excursion to the right is the peak of the spike (28 mV. overshoot). The large spot at the centre recorded the outside potential (zero) when the electrode was dislodged by the twitch. (The small peaked curve in the centre is the transient of the electrode pull-out; neither it nor the high-frequency hash on the entire record is significant.) The various regions of the phase plane record chosen for the analysis described in the text are labelled, and the arrows indicate the approximate boundaries of each region. This particular record was rotated slightly clockwise by a 3° phase-shift introduced by the differentiating network, and the inflexion point of the leading edge (at the top of the record) seems to occur at smaller membrane potentials than might be expected. It was possible, after analytical functions were fitted to various portions of the record, to obtain a description of the action potential in terms of several differential equations; that is, a set of functions where $dV/dt = f(V)$. These were integrated mathematically for $V = f(t)$, which in turn were differentiated successively for dV/dt and d^2V/dt^2 as $f(t)$, the functions required for the solution of the cable equation. It was necessary to separate the record by trial and error into several regions as indicated in Fig. 1, and to fit individual functions to each section, since no single function has been found which describes the entire course of the action potential. (Regions a and d are linear, b is closely fitted by a parabolic expression, and c by a modified cubic.) Once these functions were obtained from each record, they were integrated and differentiated as described, and yielded a set of functions suitable for direct substitution into the cable equation. Taking t as the independent variable, V and I_i were calculated point by point every 10–50 μsec. throughout the spike potential. The calculations for one record take about 2 days to complete, and this article summarizes results obtained in separate experiments from six typical fibres.

The list of fitted functions and derived equations describing the muscle spike-potential is lengthy (eighteen in all) and will not be presented here. However, the equation for region a is of particular importance and will be discussed briefly. As the action potential shown in Fig. 1 invaded the recording site, the advancing foot which develops exponentially in time[1] was necessarily transformed in the phase plane into a straight line of slope k_1 (ref. 2). It is suggested in refs. 1 and 2 that k_1 is equal to $(2RCs^2/a)$. The cell constants are thus obtained directly from this one portion of the phase plane record and permits a revision of (1) to:

$$I_i/C = (d^2V/dt^2)/(k_1) - dV/dt \qquad (2)$$

After selecting a value of 6μF./cm.² for the membrane capacitance from the average of published data, all calculated derivatives were corrected to give the membrane ionic current density I_i in m.amp./cm.², as illustrated in Fig. 2; also a portion of the spike-potential reconstructed in its usual form from the

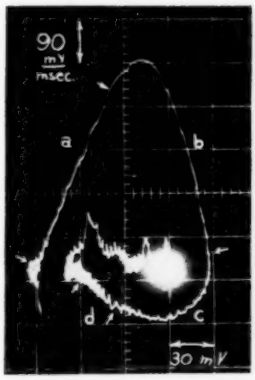

Fig. 1. Propagated action potential of a single surface fibre of the frog sartorius muscle recorded by a penetrating micro-electrode and displayed in the phase-plane
Conditions: 24° C., sodium chloride 110 mM, potassium chloride 2·5 mM, calcium chloride 4 mM, and phosphate buffer 1 mM at pH 7·3

differential equations is shown. Fig. 2 represents a reconstruction of the spike potential and membrane ionic current obtained from the phase-plane record of Fig. 1. (Time in msec. Inward current in m.amp./cm.² is plotted as positive.) Calculations were carried to within a few mV. of the beginning of the decay of the after-potential. Since the time-constant of the after-potential is close to or equal to that of resting membrane, it would appear that the conductances of the active membrane have subsided, and the remainder of the action potential is not relevant to the present work. It should be emphasized that, as a result of setting appropriate boundary conditions for the equations describing each region of the phase plane record, the derivatives and hence the potential and the ionic current are continuous in time across each junction point and throughout the entire portion of the reconstructed spike-potential.

The largest ionic current appears to be associated with the upstroke of the muscle fibre spike-potential,

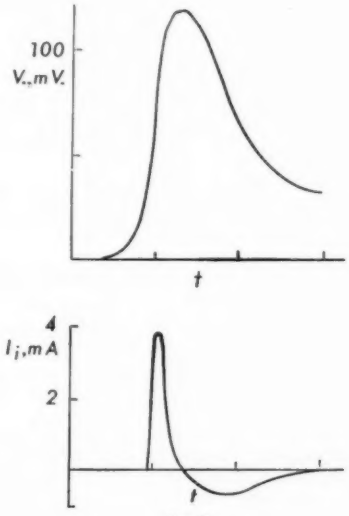

Fig. 2

as in the squid giant axon, and lasts somewhat less than 0·5 msec. at room temperature. Graphical integration of Fig. 2 predicts a net entry of positive charge during initial portions of the spike of about 7×10^{-12} mole/cm.^{2} followed by a net exit of about 6×10^{-12} mole/cm.^{2} during the terminal phase. These charges should balance, and the discrepancy is attributed to the difficulty involved in fitting a cubic equation to region *c* which is largely generated by the outward currents. Region *b* can be fitted more accurately and more confidence is placed in the corresponding portion of the inward current plotted against membrane voltage in Fig. 3. The solid curves plot the relation between the inwardly directed membrane ionic current/cm.^{2} and the membrane voltage during the upstroke of two spike-potentials obtained from the same muscle under different conditions. The largest current was obtained when the muscle was immersed in a Ringer fluid containing 110 m*M* sodium chloride while the other was obtained after the concentration of sodium chloride was reduced to 55 m*M* by replacement with choline chloride. The dashed lines are extrapolated to the potential at which the ionic currents would equal zero. The calculation for the magnitude of ionic current was based on the assumption of a membrane capacitance of 6 μF./cm.^{2}. The actual value of the capacitance enters the calculations only as a scale-factor for the ordinate and will not affect the extrapolation to + 37·5 mV. (110 m*M* sodium chloride) or + 22·5 mV. (55 m*M* sodium chloride). Note that the firing potential at − 45 mV. is not affected by the concentration of sodium ions. Although the method in itself cannot distinguish between currents carried by sodium or potassium ions, the results which followed experimental alterations of the Ringer fluid suggest that the sodium ion may carry a significant fraction of the current during the upstroke of the muscle fibre spike. For example, as shown in Fig. 3, reduction of the external concentration of sodium ion from 110 m*M* to 55 m*M* caused a decrease of 15 mV. in the extrapolated intercept as compared with the 16·5 mV. shift demanded by the resulting decrease in the sodium potential. On the other hand, analysis of other experiments clearly showed that increases of external concentration of potassium ions from 2·5 m*M* to 10·5 m*M* or of concentration of calcium ions from 1 m*M* to 10 m*M* have a negligible effect on this intercept, although the ionic current magnitudes were significantly modified. While these latter ions might be expected to alter the membrane conductances, they should not affect the sodium potential. In addition, the approximate, extrapolated zero-current potentials from the five records obtained from fibres immersed in 110 m*M* sodium chloride Ringer fluid range from + 38 to + 42 mV., and when substituted into the diffusion potential equation predict internal concentrations of sodium ions of 22–25 m*M*. These records were obtained from fibres isolated for at least 1 hr., and these concentrations seem quite consistent with published data.

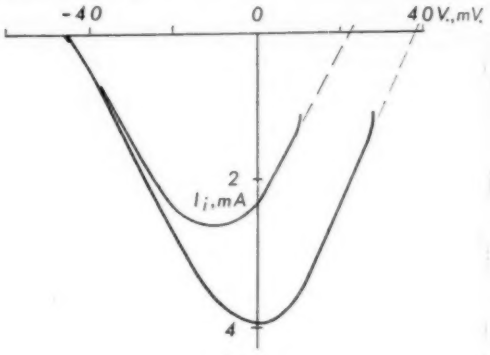

Fig. 3

Because the intercept at zero current behaves as described it has been tentatively assumed that this may be the sodium potential. If so, the ionic currents associated with this portion of the spike-potential should be carried predominantly by the sodium ion. It is recognized that the behaviour of this part of the voltage-current plot may be a fortuitous result of the chosen functions and this is being investigated.

This work has been aided by grants from the National Institutes of Health (*B*–861 and *B*–3272) and from the McCandless Fund of Emory University.

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ENLARGEMENT OF SALIVARY GLAND IN MICE TREATED WITH ISOPROPYLNORADRENALINE

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SELYE *et al.*[1] have recently reported that large doses of *iso*propylnoradrenaline (*iso*proterenol) stimulate the growth of the salivary glands of the rat. In view of the similar sex dimorphism and the similar response of the submandibular glands of both rats and mice to sex hormones and thyroxine[2], the effect of this catechol amine on the glands of mice has been investigated.

Nineteen male albino mice of the Parkes strain (mean initial body-weight 30 gm., range 28–34 gm.) were used; 7 served as controls and the others were injected twice daily intraperitoneally with *iso*propylnoradrenaline sulphate (40 mgm. per 100 gm. body-weight in 0·2 ml. of water). On the fifth day of treatment, five of the animals died. The dose was reduced to 30 mgm. per 100 gm. body-weight and the treatment continued for a further two days. On the eighth day the control animals and the survivors of the treated group were injected subcutaneously with 4 μc. of carrier-free ^{131}I-iodide in a volume of 0·1 ml. and killed 2 hr. later. The external lacrimal, sublingual, submandibular and parotid glands were removed, weighed rapidly to the nearest 0·1 mgm. and one gland of each pair placed in 10 per cent neutral formol-saline for fixation. A serum sample was obtained and the second sub-

mandibular gland was dissolved in 3 *N* sodium hydroxide. Radioiodine contents were measured in an *M*6 liquid Geiger counter and the concentration ratio (counts/sec./100 mgm. gland: counts/sec./100 mgm. serum) was calculated.

The results obtained are shown in Table 1. Significant increases in the weight of the submandibular and parotid glands were observed in mice dying after 5 days treatment and in mice killed on the eighth day. The further increase between the fifth and eighth days was significant in the case of the parotid gland but not in that of the submandibular gland. In confirmation of the findings of Selye *et al.*[1], the weight of the external lacrimal gland was not affected by this treatment. The sublingual gland weight was not increased in these mice and the submandibular gland weight at the eighth day was only 150 per cent of the control value as compared to a parotid weight of 550 per cent of the control value. Selye *et al.*[1] state that the parotid, submandibular and sublingual glands were enlarged to approximately the same degree in female rats treated with a similar dose of *iso*propylnoradrenaline for 17 days, but do not give figures for the individual glands. The difference could be due to the difference in species, sex or duration of treatment.

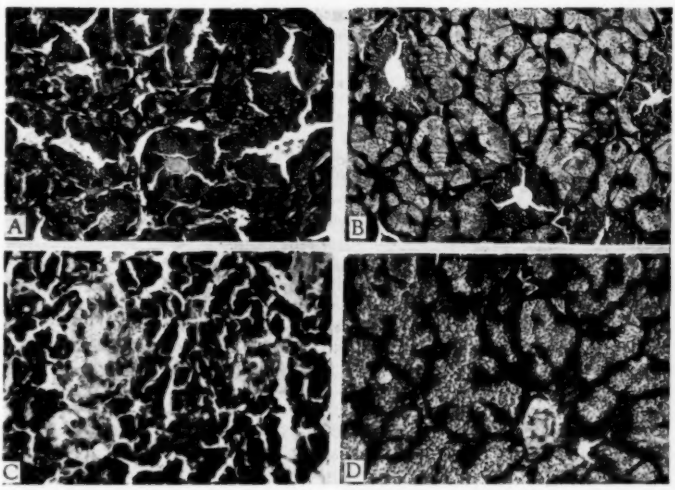

Fig. 1. 7-μ sections, stained hæmatoxylin and eosin. (× *c.* 200.) *A*, Normal mouse submaxillary gland. Note predominance of granulated tubules in the field; *B*, treated mouse submaxillary gland. Note increase in size of acinar cells. Granulated tubule size maintained. Cell granules are prominent; *C*, normal parotid gland. Small basophilic acinar cells; tubule cells show well marked striations with round central nuclei; *D*, treated parotid gland. Note acinar cell enlargement with displacement of nuclei. Ducts compressed with loss of basal striations in cells

Histological examination (paraffin embedding, 7-μ sections stained with hæmatoxylin and eosin) of the submandibular and parotid glands of all the control mice and of all those killed after 8 days treatment revealed marked differences. The most striking feature of both submandibular and parotid glands was a great increase in the size of the acinar cells; the cytoplasm was filled with basophilic secretory granules; the nuclei often appeared compressed and were displaced to the periphery of the acini. Intercellular canaliculi were prominent and contained material with the same staining properties as that seen in the major intralobular ducts. Intercalated ducts which are not obvious in the normal gland were prominent in the treated animals because of the distension of their lumina by secreted material. The condition of the major intralobular ducts differed in the two glands; the striated ducts of the parotid were found to be compressed when the diameter was measured from the base of the tubule cells. The lumen, however, was dilated with basophilic secretion; the tubule cells themselves were flattened, the normally round, central nuclei appeared as ellipsoids with their long axes parallel to the length of the tubule. The normal basal striations of the cytoplasm were much less obvious and in some cases absent. The convoluted granular tubules (serous tubules) of the submandibular gland which predominate in the normal male gland were less obvious in the treated animals because of the great increase in size of the acinar cells. Examination of individual tubules did not show any change in external diameter or in the size of individual tubule cells comparable with the changes in acinar cells. A possible increase in the number of large, intensely eosinophilic granules was suspected in some of the glands from treated animals; but considerable variation within both control and experimental groups and within the same gland made a decision on this point difficult. Sections stained by the periodic acid–Schiff method and counterstained with aniline blue and orange *G* showed that some acinar cells in the normal parotid contained periodic acid–Schiff-positive granules lying at the luminal borders of the cells. A faint positive reaction was observed diffusely in the cytoplasm of the majority of acinar cells in the treated gland. The normal submandibular acinar cells showed areas of periodic acid–Schiff-positive material in the cytoplasm; in the treated gland the acinar cell cytoplasm was uniformly positive; the contents of the ducts gave a strong positive

Table 1. GLAND WEIGHTS (MGM.) AND THE SUBMANDIBULAR GLAND/SERUM CONCENTRATION RATIOS FOR IODINE-131 IN NORMAL AND *ISO*PROPYLNORADRENALINE-TREATED MICE

Figures are means ± S.E.M. Not significant (N.S.) indicates a value for P of greater than 0·05 in the t test for the significance of the difference of group means

	No.	External lacrimal	Sublingual	Parotid	Submandibular	Concentration ratios iodine-131
Controls	7	27·4 ± 2·1	25·5 ± 1·8	88·4 ± 7·7	152·4 ± 6·6	3·34 ± 0·24
Died after 5 days treatment	5	21·1 ± 2·0	31·4 ± 4·9	315·8 ± 22·1	197·8 ± 5·8	—
P values for difference from controls		N.S.	N.S.	< 0·001	< 0·001	—
Killed on eighth day	7	25·1 ± 1·6	26·5 ± 1·6	474·9 ± 19·4	228·3 ± 16·9	3·90 ± 0·64
P values for difference from controls		N.S.	N.S.	< 0·001	< 0·01	N.S.
P values for difference from animals dying at fifth day		N.S.	N.S.	< 0·001	N.S.	—

reaction. The mitotic proliferation reported by Selye *et al.*[1] in the rat was not seen in these mouse glands. Mitotic figures were searched for, but were extremely rare.

The histological picture is interpreted as showing an intense stimulation of the glands resulting in an increase in size of the acinar cells which are swollen with secretory material; many of the intralobular duct lumina are also distended with secretion. There is no histological evidence of a comparable stimulation of duct cells in the parotid glands or in the intercalated ducts, striated intralobular ducts or interlobular ducts of the submandibular gland. There is some evidence of stimulation of the cells of the serous tubules, which are not compressed like the cells of other ducts, and many show an increase in the number and size of their secretory granules. The gland/serum concentration ratio for radioiodide is not significantly altered (Table 1). This observation may be related to the histological evidence of stimulation of the convoluted granular tubules, as these are the elements in the gland which are thought to be responsible for the high concentration of iodide in the saliva and in the gland itself relative to the serum[2]. The maintenance of the normal gland : serum concentration ratio, despite the marked increase in the acinar cell mass, is also suggestive of a possible stimulation of the serous tubule cells.

No evidence of hyperplasia of the glands was seen in the present experiments, nor is it necessary to invoke this to explain the increase in weight. The relative width of the acinar cells in the normal and treated glands was measured in representative sections and was found to be in the range of 1 : 1·6–1 : 1·8. Assuming the cells to be cuboidal and the specific gravity to be unchanged, such an increase in size in cell dimensions would result in a weight ratio of about 1 : 4·9, roughly what was found in the parotid glands, where acinar cells predominate over tubules. The smaller increase in weight in the submandibular glands, despite an equally striking increase in the size of the acinar cells, may be due to the far greater proportion of the normal gland weight that is contributed by the tubules which do not increase in size during treatment with large doses of *iso*propylnoradrenaline. Although the weights of the sublingual and external lacrimal glands were not increased, histological examination showed that these glands, too, had been affected. There was evidence of a much less-marked and more variable increase in size of the acinar cells with the accumulation of secretory material within the cells and in the duct lumina.

The changes observed in the salivary glands of these mice are consistent with an intense, but not unexpected, stimulation of acinar cell secretory activity by these very large doses of *iso*propylnoradrenaline. This, rather than a sialoadenotrophic action of this compound, such as has been reported in rats[1], appears to be the most probable explanation for the dramatic changes in weight observed.

My thanks are due to Prof. J. Orr, Dr. F. Jacoby and Dr. Kathleen Hall for their advice, and to Mr. D. Varley and Miss Rita Hore, who prepared the sections and photomicrographs for me.

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STIMULATION OF TURNIP YELLOW MOSAIC VIRUS PROTEIN PRODUCTION BY 2-THIOURACIL

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THE uracil analogue, 2-thiouracil, reduces the rate of multiplication of several plant viruses[1]. When tobacco mosaic virus multiplication is inhibited by 2-thiouracil, the analogue is incorporated into the virus ribonucleic acid (RNA)[2]. Both whole virus containing 2-thiouracil and RNA prepared from it by the phenol method[3] appear to be less infectious than normal virus. In the experiments reported here we have found that, while 2-thiouracil suppresses multiplication of turnip yellow mosaic virus (TYMV) nucleoprotein, it causes a spectacular increase in the production of virus protein shells containing no RNA. The analogue is not incorporated into the RNA of complete virus in detectable amounts.

The strain of TYMV used and the methods of inoculation and culture in Chinese cabbage plants have been described[4]. We have found that Chinese cabbage plants are much less susceptible to gross leaf injury and inhibition of growth by 2-thiouracil than many other species, such as tobacco. Plants to be treated with 2-thiouracil were sprayed daily with a 0·001 *M* solution of the analogue, controls being sprayed with water. To reduce variability in the sample, groups of 6–18 plants were used for each treatment and one or more disks were taken from each leaf and pooled at each time of sampling. In most experiments the disks were ground immediately after sampling and the sap pressed through muslin. The sap was then examined in a Spinco model *E* analytical ultracentrifuge either without further treatment or after heating at 55° for 5 min.—a treatment which destroys the integrity of the plant ribosomes. Sap from healthy Chinese cabbage plants sprayed with 2-thiouracil and heated in this way shows no peaks sedimenting at the rates of the virus protein or nucleoprotein. In some of our early tests samples of leaf were frozen before examination. We abandoned this procedure, however, since we found that freezing may convert a substantial proportion of the empty protein shell fraction and a lesser amount of the nucleoprotein to a heterogeneous material sedimenting at about the same rate as the plant ribosomes.

Quantitative estimates of the concentration of components were made by measuring areas under the schlieren curves. Areas were corrected for radial dilution, but not for the Johnston–Ogston effect. Difficulty in assessing the correct base line in the

schlieren patterns from crude plant extracts leads to significant ambiguity when the areas of the peaks are small. The base line is easier to determine when heat-clarified sap is used, but heat-treatment leads consistently to a slight reduction in the area of the empty protein shell peak. We do not yet know whether this reduction is due to error in base line determination or to destruction of a small proportion of the empty shells. However, this source of error is negligible for the major effects described here. Areas were translated into concentrations in mgm./ml. using experimentally determined factors for the virus protein and nucleoprotein. In some experiments an independent estimate of the virus nucleoprotein material was made by the serological–chromatographic procedure[5].

Earlier experiments[6] in which rather crude methods were used, had suggested that the ratio of virus protein to nucleoprotein in plants infected with TYMV was fairly constant, but our examination of heat-clarified sap in the ultracentrifuge has shown that this is not so. In normal infections the virus nucleoprotein fraction is the first detectable component (6–8 days after infection in inoculated leaves and 9–11 days in systemically infected leaves). The virus protein peak appears a few days later, and for several days thereafter the virus protein/nucleoprotein ratio rises, reaching a maximum of about 0·4–0·6 and then falling steadily to about 0·1–0·3.

Fig. 1 illustrates the substantial increase in the area of the virus protein peak that is brought about by appropriate treatment of plants with 2-thiouracil. Our evidence that the material constituting this increased peak is largely or entirely TYMV protein is as follows: (1) it has the expected sedimentation coefficient; (2) the material composing the peak is as stable to heating at 55° as the virus protein peak from untreated plants; (3) the peak, along with the virus nucleoprotein peak, is quantitatively removed by the addition of a slight excess of antiserum prepared by the injection of rabbits with highly purified TYMV B_1 nucleoprotein[4]; (4) both the ethanol-ammonium sulphate[7] and *p*H 4·8 (ref. 4) procedures for purifying TYMV are known to be effective in removing plant proteins and nucleoproteins. When TYMV is isolated by either of these methods from sap such as that shown by Fig. 1*A*, the ratio of the virus protein to nucleoprotein peaks remains substantially unchanged through all steps in the isolation; (5) when purified virus preparations made from infected plants suitably treated with 2-thiouracil are fractionated by sedimentation into dense cæsium chloride solutions[4], the opalescent band sedimenting in the position of the empty protein shell is greatly increased in amount relative to the B_1 nucleoprotein. The material isolated in this way has an ultra-violet absorption spectrum indistinguishable from the normal virus protein.

Under conditions where 2-thiouracil causes a large increase in the content of empty virus shells, it has no detectable effect on the areas of the plant ribosome peak, or the fraction I (ref. 8) protein peak.

The effect of 2-thiouracil treatment on the amounts of TYMV protein and nucleoprotein produced in the leaf depends strongly on the time after infection at which treatments are begun (Fig. 2). If spraying is started within a few days after inoculation, nucleoproduction is strongly suppressed, while protein production is not markedly affected. This leads to a large increase in the protein/nucleoprotein ratio, although there is a net decrease in the rate of production of total virus protein (empty protein shell + protein in the nucleoprotein fraction). If treatment is begun when the virus infection is well established, 2-thiouracil causes a striking rise in the production of empty protein shells. The production of nucleoprotein is not as strongly depressed as when treatments are started earlier. The net effect is a substantial increase over untreated plants in the content of total virus protein material.

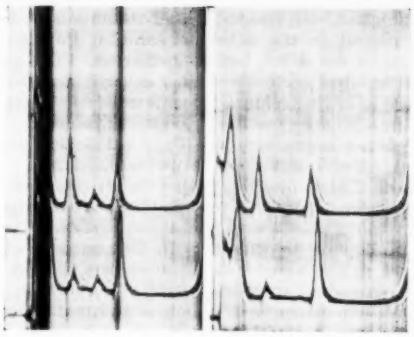

Fig. 1. Schlieren diagrams of expressed sap from Chinese cabbage plants infected with TYMV. *A*, untreated sap after centrifugation at 35,600 r.p.m. for 14 min.; *B*, sap heated to 55° for 5 min. and then centrifuged at 31,410 r.p.m. for 21 min. Plain cell (lower pattern) contains sap from control plants. Wedge cell (upper pattern) contains sap from plants sprayed daily with 2-thiouracil for 10 days. For Fig. 1*A* peaks in order from left are: (*a*) fraction I plant protein; (*b*) TYMV empty protein shell; (*c*) plant ribosomes; (*d*) TYMV nucleoprotein

Spraying infected plants daily with 0·001 *M* uracil beginning 13 days after inoculation causes an increase in the rate of production of virus nucleoprotein, but the protein/nucleoprotein ratio is not affected. Thus the availability of uracil may be a factor limiting TYMV production. If, however, plants are sprayed with a mixture of 2-thiouracil and uracil (both 0·001 *M*), uracil does not annul the effect of 2-thiouracil. Rather the production of excess protein shells reaches substantially higher levels than with 2-thiouracil alone. The stimulation of virus protein synthesis can, however, be halted by washing the leaves with water and stopping application of 2-thiouracil.

Experiments in which plants were supplied with sulphur-35 as inorganic sulphate, as described in ref. 4, suggest that the rapid increase in empty protein shells caused by 2-thiouracil is due to the formation of new protein rather than 'polymerization' of pre-existing sub-units of the virus shell. For example, in one test, two virus-infected plants, one

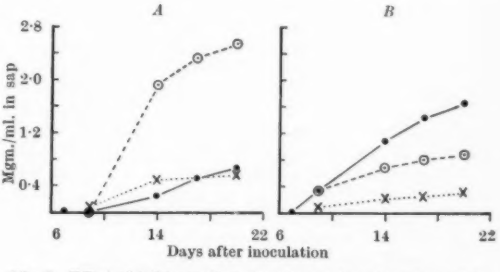

Fig. 2. Effect of 2-thiouracil on TYMV protein and nucleoprotein production. *A*, virus protein shells; *B*, protein in virus nucleoprotein; ●, control sprayed with water; ×, sprayed daily with 2-thiouracil beginning 1 day after inoculation; ⊙, sprayed daily with 2-thiouracil beginning 9 days after inoculation

of which had been treated with 2-thiouracil for 3 days, were placed in the sulphate solution labelled with sulphur-35 for 4 hr. before gathering. The protein in the isolated protein and B_1 nucleoprotein fractions had the following relative specific activities (expressed as c.p.m./mgm. protein): control protein, 7,570; control nucleoprotein, 4,570; 2-thiouracil protein, 42,500; 2-thiouracil nucleoprotein, 1,200. This differential labelling, together with the observed variations in the ratio of protein shells to nucleoprotein in untreated plants and the accumulation of protein shells following treatment with 2-thiouracil, all lend support to the view[4,9] that the empty protein shells are precursors of complete virus.

In all experiments in which concentrations of the virus components were measured in expressed sap, the nucleoprotein fraction, while consisting largely of the B_1 component[4], also included the B_2 and possibly the B_0 components. We have found that, by sedimenting purified virus preparations in the analytical centrifuge at high concentrations (10 mgm./ml.) in a medium of cæsium chloride of density = 1·36, the B_2 component migrates, as a peak with measurable area, ahead of the B_1 component. The concentration of B_1 in the preparation is estimated from the area of the peak obtained in a second run at lower concentration (2–3 mgm./ml.) in 0·1 *M* sodium chloride. Using this procedure, we have been able to estimate the ratio B_2/B_1. This ratio was consistently less in preparations from plants where empty protein shell was accumulating rapidly and where the rate of nucleoprotein production was reduced by treatment with 2-thiouracil. For example, in a control virus preparation where the ratio of protein to nucleoprotein was 0·25, the ratio B_2/B_1 was 0·042. The corresponding figures for a preparation from plants treated with 2-thiouracil for 4 days were protein/nucleoprotein 0·88; B_2/B_1 0·029. This reduction in the ratio B_2/B_1 would be consistent with the suggestion[4] that B_2 may be a stage in the assembly of complete virus from empty protein shells.

2-Thiouracil does not appear to be incorporated into the virus RNA. In one test where plants were treated with 2-thiouracil labelled with sulphur-35 (Amersham code *SJ*50) no significant radioactivity could be detected in either whole purified virus preparations or in the isolated B_1 component under conditions in which we would have detected incorporation of 2-thiouracil equivalent to 0·01 per cent of the virus uracil. In this experiment the ratio protein/nucleoprotein at the beginning of treatment was 0·19 and at time of collecting (after 8 days) was 0·73.

The reason why 2-thiouracil causes protein shells to accumulate is at present quite obscure. The phenomenon bears at least a superficial resemblance to the action of proflavine on certain viruses attacking *Escherichia coli*[10]. 2-Thiouracil may have its effect in part either by inhibiting virus RNA synthesis or, if virus RNA is made, by preventing its incorporation into the protein shell. However, neither of these effects could explain the appearance of a greater total of virus protein material in leaves treated with 2-thiouracil. Lack of incorporation of the RNA might be due to the production of either faulty RNA or faulty virus protein shells or both of these. As yet, we have no evidence as to whether the protein which accumulates is biologically functional or not.

It should be possible to determine whether free virus RNA accumulates at a rate equivalent to the increase in protein shells since after treatment for a few days with 2-thiouracil the amounts present would be about 0·5 mgm. of RNA per gm. fresh weight of leaf.

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ROOT TEMPERATURE AND SYMBIOTIC NITROGEN FIXATION

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THE effect of physical environment on nodule formation and symbiotic nitrogen fixation has received little attention, although such factors as soil and air temperatures, light intensity and moisture conditions appear to be important. Investigation of the effects of these factors has begun, with the initial emphasis on effects of root temperature on symbiotic nitrogen fixation by *Trifolium subterraneum* (subterranean clover).

Hely (personal communication) has observed that during winter periods nodulated clover plants make poor growth compared with plants receiving combined nitrogen. Meyer and Anderson[1] have shown that moderately high temperatures specifically inhibit nitrogen fixation, although Mes[2] suggested that their conclusions should be restricted to subterranean clover. The aim of this investigation was to find the most effective combinations of host and bacterial strain suitable for the various ranges of environmental conditions found in the field. This article reports two experiments with two varieties of *T. subterraneum* inoculated with each of two strains of *Rhizobium trifolii*.

The plants were grown on mineral nutrient agar, free from available combined nitrogen, the enclosed tube technique[3] being modified so that only the roots grew inside the tube. Two days after sowing surface-sterilized pre-germinated seeds, the plants were either inoculated or left uninoculated as controls; 13 days later the inoculated plants were well nodulated. At this time a uniform population of plants was selected, and ammonium nitrate added to the

controls. One-fifth of the plants were collected for initial nitrogen-level determinations, and the remainder transferred to a controlled environment unit in which the tubes were suspended in temperature-controlled water-baths (temperature variation ± 0·3 deg. C. in the tubes). The source of light was a bank of 'Warm White' fluorescent, and incandescent, tubes giving an intensity of 1,000 ft.-candles, and a photoperiod of 16 hr. For all plant tops the phototemperature was 22–23° C., and the nyctotemperature 10–11° C. The tubes were re-filled with sterile nutrient solution when necessary and ammonium nitrate added to the controls at regular intervals to give the following amounts of combined nitrogen: 4 mgm. for root temperatures 5° and 9·5° C., and 6 mgm. for 14° and 18° C. in experiment 1; 9 mgm. for 18°, 22°, 26° and 30° C. in experiment 2. The ten replicates of each treatment were collected after growth for 20 days in the unit, and the dry weights and total nitrogen determined.

The values in Table 1 express nitrogen fixed during the temperature treatments as a percentage of the combined nitrogen assimilated by the control plants; these values provide an index of the effectiveness of the symbiosis[4]. In experiment 1, the Tallarook controls at 18° C. appeared to be slightly deficient in nitrogen, although the amount assimilated was less than that added. For experiment 2, higher levels of ammonium nitrate were added to the controls, but the amounts assimilated were probably excessive, as shown by: (1) a percentage nitrogen of 4·3–4·7 in all control treatments compared with a normal level of 3–3·5 per cent; and (2) a close similarity in total dry weight of the controls and the most effectively nodulated treatments (Tallarook inoculated with *TA*1). The excessive assimilation of combined nitrogen probably explains the generally lower values for the index of effectiveness in experiment 2, relative to experiment 1, in Table 1.

At root temperatures of 5° and 9·5° C., Tallarook inoculated with strain *TA*1 is able to fix nitrogen as effectively as the controls can assimilate combined nitrogen, whereas the remaining three combinations of host and strain are less effective. As the temperature rises, the effectiveness of these three associations improves. Above 22° C., the effectiveness of nitrogen fixation by strain *NA*30 on both varieties is reduced considerably, while the effectiveness of strain *TA*1 remains comparatively constant. The results obtained with strain *NA*30 are similar to those reported by Meyer and Anderson[1], who also used this strain (personal communication). However, the results with strain *TA*1 suggest that their postulate that moderately high temperatures specifically inhibit symbiotic nitrogen fixation requires further elucidation.

Table 1. Effectiveness of Symbiotic Nitrogen Fixation and Combined Nitrogen Uptake (mgm. Ammonium Nitrate – Nitrogen/Plant) at Different Root Temperatures

Root temp. (°C.)	Uptake of ammonium nitrate		Index of effectiveness			
			Variety Tallarook		Variety Dwalganup	
	Tallarook	Dwalganup	Strain *TA*1	Strain *NA*30	Strain *TA*1	Strain *NA*30
Exp. 1						
5·0	0·99	0·90	97·6	49·7	42·6	50·0
9·5	2·67	2·07	104·2	72·8	58·4	67·7
14·0	4·46	3·58	90·2	79·7	65·1	75·8
18·0	4·55	3·76	111·0	86·4	63·8	82·8
Exp. 2						
18·0	5·96	4·83	72·4	56·1	45·1	57·2
22·0	9·05	6·89	74·3	51·0	48·7	49·1
26·0	7·41	6·54	85·8	32·7	39·7	35·7
30·0	7·00	2·68	74·9	9·9	58·8	18·8

The index of effectiveness is derived from

$$\frac{\text{nitrogen fixed}}{\text{ammonium nitrate uptake}} \times 100$$

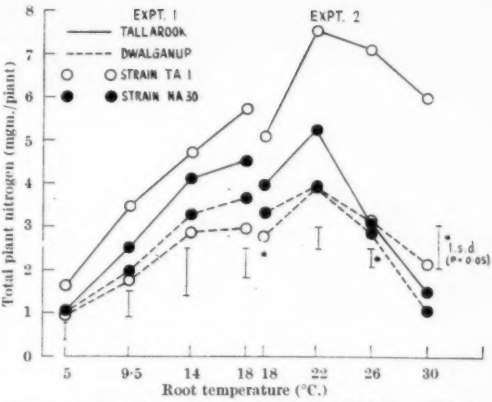

Fig. 1. Effect of root temperature on total plant nitrogen

*Owing to significant differences between error variances, no L.S.D. values are shown for the treatment means at 18° C., nor for the Tallarook means at 26° and 30° C. in experiment 2.

It would appear that the temperature at which nitrogen fixation is affected, relative to plant growth on combined nitrogen, may depend on the bacterial strain, or at least on the host × strain combination. However, it is possible, as found with these experimental conditions, that fixation of nitrogen by some combinations of host and strain is relatively insensitive to higher temperatures, when compared with the uptake of combined nitrogen; for example, Dwalganup controls made poor growth at 30° C., yet the index of the effectiveness of nitrogen fixation by this host, inoculated with *TA*1, remains similar to that at 18–26° C.

Jones and Tisdale[5] showed that four species of nodulated legumes differed in their tolerance of high soil temperatures. Mes[2] observed that maximum nitrogen fixation by several species of nodulated legumes was attained at different day temperatures, and also at different night temperatures. In neither case were the effects of bacterial strain examined. In the present investigation, temperature influenced the relative effectiveness of two strains of *Rhizobium trifolii* on two varieties of the one host species (Fig. 1). With the variety Tallarook, there is a marked divergence in the level of total plant nitrogen for the two strains of *Rhizobia* as the root temperature rises above 22° C. In addition, there is a temperature × strain interaction with the variety Dwalganup, with *NA*30 being the better strain below 22° C., and strain *TA*1 superior at 30° C. This reversal of the order of effectiveness of the two strains on Dwalganup has been observed repeatedly.

The demonstration of temperature × strain, and host × strain, interactions within the species *T. subterraneum* indicates that these three factors should be considered in the selection of strains of *Rhizobia*, if the full potential of the legume-rhizobia symbiosis is to be attained.

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LETTERS TO THE EDITORS

COSMOLOGY

An Unsteady Universe with Excess of Charge

RECENTLY, Lyttleton and Bondi[1] put forward the suggestion that the outflow of matter in the universe is caused by a net charge on the hydrogen atom, and that in order for the universe to remain steady in the large, there is creation of matter. This analysis was simplified by me[2] with the aid of Watson's creation potential[3].

Suppose, however, that the universe is not steady, but is uniform, being composed of hydrogen atoms of mass m and charge e. Following the arguments of Milne[4], if the velocity of any particle, the position vector of which with respect to the origin is $\mathbf{r}$, is $\mathbf{r}/t$ each particle in a Newtonian system can equally be regarded as the centre of the universe. The associated acceleration is:

$$\frac{d\mathbf{v}}{dt} = (\mathbf{v}.\nabla)\mathbf{v} + \frac{\partial \mathbf{v}}{\partial t}$$

$$= (\alpha^2 - \alpha)\frac{\mathbf{r}}{t^2}$$

The proof is given by Milne for the case $\alpha = \frac{2}{3}$, and the extension is obvious.

Maxwell's modified equations are[3]:

$$\nabla \times \mathbf{E} + \frac{\partial \mathbf{B}}{\partial t} = 0, \quad \nabla . \mathbf{B} = 0,$$

$$\nabla \times \mathbf{H} - \frac{\partial \mathbf{D}}{\partial t} = \mathbf{J} - \frac{1}{\mu_0}\nabla N, \quad \nabla . \mathbf{D} = \rho + \varepsilon_0 \frac{\partial N}{\partial t}$$

If a solution be taken for which $\mathbf{H}$ is zero as previously[2]:

$$\nabla \times \mathbf{E} = 0, \quad -\frac{\partial \mathbf{D}}{\partial t} = \mathbf{J} - \frac{1}{\mu_0}\nabla N,$$

$$\nabla . \mathbf{D} = \rho + \varepsilon_0 \frac{\partial N}{\partial t}$$

Eliminating N, it follows that:

$$\nabla(\nabla . \mathbf{D}) - \mu_0\varepsilon_0 \frac{\partial^2 \mathbf{D}}{\partial t^2} = \nabla \rho + \mu_0\varepsilon_0 \frac{\partial \mathbf{J}}{\partial t}$$

$$= \nabla \rho + \mu_0\varepsilon_0 \frac{\partial}{\partial t}(\rho \mathbf{v})$$

$\mathbf{v}$ being the flow velocity of the electric charge density.

If m be the mass of a hydrogen atom, then:

$$m\frac{d\mathbf{v}}{dt} = e^* \mathbf{E} = e^* \frac{\mathbf{D}}{\varepsilon_0}$$

where:

$$e^* = e\left(1 - \frac{\gamma m^2 4\pi\varepsilon_0}{e^2}\right)$$

that is, if the electric repulsion between two particles is greater than the gravitational attraction between them, e^*/e is positive, and vice versa.

It follows, therefore, that:

$$\mathbf{D} = \frac{\varepsilon_0 m}{e^*}\frac{d\mathbf{v}}{dt} = \frac{\varepsilon_0 m}{e^*}(\alpha^2 - \alpha)\frac{\mathbf{r}}{t^2}$$

This field satisfies $\nabla \times \mathbf{E} = 0$ identically.

On the assumption that the universe is uniform in the large, $\nabla \rho$ vanishes; also $\nabla . \mathbf{r} = 3$ and so $\nabla(\nabla . \mathbf{r}) = 0$.

$$\mu_0\varepsilon_0 \frac{\partial}{\partial t}\left(\rho\, \alpha \frac{\mathbf{r}}{t}\right) = \frac{\varepsilon_0 m}{e^*}(\alpha^2 - \alpha). - \mu_0\varepsilon_0 \frac{6\mathbf{r}}{t^4}$$

whence:

$$\rho = \frac{2\varepsilon_0 m(\alpha - 1)}{e^* t^2}$$

The rate of production of charge per unit volume is given by:

$$\nabla . (\rho \mathbf{v}) + \frac{\partial \rho}{\partial t} = \nabla . \left(\frac{2\varepsilon_0 m(\alpha - 1)\alpha \mathbf{r}}{e^* t^2}\right) - \frac{2\varepsilon_0 m(\alpha - 1).2}{e^* t^2}$$

$$= \frac{2\varepsilon_0 m}{e^* t^2}(\alpha - 1)(3\alpha - 2) = \frac{\rho}{t}(3\alpha - 2)$$

The rate of creation of atoms per unit volume is, of course, obtained by dividing by e. The cases where the production-rate are zero $\alpha = 1$, and $\alpha = \frac{2}{3}$. In the first case, the acceleration of the nebulæ $\frac{d\mathbf{v}}{dt}$ is zero[5] which would correspond here to the electric repulsion being exactly equal and opposite to the gravitational attraction, thus balancing it out. The second case corresponds to Milne's Parabolic Universe[6].

The recession-rate of the nebulæ is given also by $\mathbf{v} = \frac{\mathbf{r}}{\tau}$, where τ is the inverse of Hubble's constant. Thus, the time at present is $\alpha\tau$, and the present rate of production of charge density is $\rho/\tau\left(3 - \frac{2}{\alpha}\right)$. Corresponding to this is a present rate of production of mass density $\frac{\rho_m}{\tau}\left(3 - \frac{2}{\alpha}\right)$ where ρ_m is the present mass density. Now

$$\rho_m = \frac{m}{e}\rho = 2\varepsilon_0 m^2 \frac{e}{e^*}\frac{\alpha - 1}{t^2}$$

and at present,

$$\rho_m = 2\varepsilon_0 m^2 \frac{e}{e^*}\frac{\alpha - 1}{\alpha^2\tau^2}$$

There is experimental evidence[7] that:

$$\frac{8}{3}\pi\gamma\rho_m\tau^2 = U^{-1}$$

where U is a number the value of which is about unity and so it follows that:

$$\frac{e^2}{4\pi\varepsilon_0\gamma m^2} = \frac{4}{3}\, U\, \frac{\alpha - 1}{\alpha^2} + 1$$

The left-hand side is the ratio of electric repulsion to gravitational attraction, and must be positive, and so $\frac{4}{3}\, U\, \frac{(1 - \alpha)}{\alpha^2} < 1$. Now e is determinate in

principle as indicated by Lyttleton and Bondi, and ρ_m and τ (and hence U) are also determinate from astronomical observations. It follows, therefore, theoretically that α can be found, and hence the present rate of production of matter.

The observations made at time t of matter at a distance R, will refer to the matter at a time $t - R/c$. It follows, therefore, that the observed mass density at a distance R is:

$$2\varepsilon_0 m^2 \frac{e}{e^*} \frac{\alpha - 1}{(\alpha\tau - \frac{1}{2}R/c)^2} = \rho_0\left(1 - \frac{R}{\alpha c\tau}\right)^{-2}$$

where ρ_0 is the local mass density at present. Also the observed recession velocity is:

$$\frac{\alpha R}{\alpha\tau - R/c} - \frac{R}{\tau}\left(1 - \frac{R}{\alpha c\tau}\right)^{-1}$$

Both these quantities, the observed mass density and the observed recession velocity, are in principle obtainable (albeit the experimental techniques are not good enough at present) and so α is obtainable in principle, and a test of this theory will be possible once the data are known with sufficient accuracy.

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ASTRONOMY

The Alpha Virginis (Spica) Nebula

An extensive ultra-violet nebula surrounding Spica has been reported by Kupperian, Boggess, and Milligan[1]. Their rocket-borne photon counters yielded an isophotal map of roughly circular extent 22° in diameter, increasing in brightness from the outside towards Spica but reaching the instrumental saturation-level for the central area. If the nebula were due to Lyman-α radiation it would be reasonable to assume that H_α should also be detectable, but no nebula has ever been detected by conventional photographic means. This may have been due to the fact that the strong image of Spica drowns the faint nebula close to the star. On the red plates of the Palomar Sky Survey, the over-exposed image of Spica covers more than 10 min. of arc, while on the blue plates it is nearly three times larger in diameter. The spectrum of Spica itself does not show H_α in emission[2].

Recently, Johnson[3] reported negative results in H_α of the nebula by means of direct and spectroscopic exposures centred on the brightest ultra-violet isophote about 10 min. of arc west of Spica.

In order to examine the area immediately surrounding the star a number of long exposures have been taken during the past 18 months by means of a novel device which might be called a 'stellar coronagraph', attached to the 30-in. refractor at Allegheny Observatory. This device, to be described in detail in a later communication, prevents the stellar image from reaching the emulsion and obscures a circular area 1 mm. (14·6 sec. of arc) in diameter at the focal plane, thus completely eliminating the over-exposed stellar image and halation pattern. To cover the range of H_α, Eastman 103*a*-*E* plus Corning 2403 filter was exposed to a limiting stellar magnitude of $m_{pg} \approx 17$. A few blue exposures on 103*a*-*O* without filter were also taken. The useful field is only 6 × 8 min. of arc with the 14·6 sec. diameter blank space at the centre. None of the exposures reveals any trace of nebulosity.

This method looks promising for detecting circumstellar envelopes and faint companions close to bright stars and should yield best results on large reflectors with large plate scales.

It is a pleasure to thank Dr. N. E. Wagman for making the telescope available for these studies.

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GEOPHYSICS

Height of Night-time *F* Layer Irregularities at the Equator

It has been established by observations at low latitudes that there exists near the equator a belt in the *F* region in which large irregularities in electron density give rise to equatorial spread *F*[1] severe radio star scintillations[2] and the scatter of radio waves[3]. Work by Hewish[4] and Briggs[5] on extra-terrestrial radio sources shows how the height of the irregularities may be inferred from observations of the diffraction pattern on the ground. But the methods used are rather indirect, and therefore subject to some uncertainty. More recently Cohen and Bowles[6], using radar techniques, arrived at an estimation of irregularities of height for equatorial locations. It is not certain that these irregularities are to be identified with those responsible for satellite and radio star scintillation.

Work on these disturbances now being pursued at University College, Ibadan, and at the University of Ghana, Accra, shows that the irregularities in the pattern on the ground are elongated in the direction of the Earth's magnetic field, that the axial ratio is greater than 6:1, and that the smaller dimension is of the order of 0·5 km. (J. R. K. and G. S. K., unpublished). These results are deduced from the study of satellite and radio star transmissions through the disturbed region. It is desirable to determine the heights of the irregularities by a direct method. The procedure adopted consists in the observation at two separated stations of the diffraction pattern produced on the ground when the radio transmissions from an Earth satellite pass through the irregularities. The velocity of the pattern over the ground is determined, and by relating this velocity to the known height and velocity of the satellite, it is possible to determine the effective height of the diffracting screen. Since the velocity of drift of the diffracting screen itself is known to be of the order of 150 m./sec. (J. R. K., unpublished) it can be neglected without introducing an appreciable error.

The observations were made at Legon, near Accra, Ghana (5° 38′ N.; 0° 11′ W.), and the satellite used

was *Tiros II* (1960 π 1). Since its apogee and perigee distances are 724 and 624 km. respectively, it is normally above the electron-density maximum in the *F* layer. The orbit is inclined to the equator at an angle of 48·6°. The signal used was the transmission on 108·03 mc./s. At each station the signal was received on a half-wave dipole, and fed to a crystal-controlled converter ahead of a Collins type *R.390A/URR* radio receiver. To achieve a satisfactory signal to noise ratio it was necessary to employ a bandwidth of 100 cycles per second. Tuning was done manually at both receivers throughout each observation. In each case the receiver *B.F.O.* was used to give a 2,000 c.p.s. note, which was transmitted via telephone cable to a central recording location. The amplitudes of the signals were then displayed on a double-beam oscilloscope and recorded photographically. A number of observations were made with an east–west base-line 1 km. in length. Later a 2·4 km. base-line was used, inclined 48° to the equator in the plane of the satellite orbit. Observations were limited to night-time passes in the period June 21–July 13, 1961.

If the irregularities occur in a thin layer at a height z and the satellite is moving horizontally at a height h and a velocity V, it can be shown that the diffraction pattern will move across the ground with a velocity $V\frac{z}{h-z}$ (ref. 7). In the case of a satellite moving in an elliptical orbit around the Earth, the expression is more complicated; but the velocity and direction of movement of the pattern on the ground can still be derived from a knowledge of the orbit. The time-shifts observed in the fading at the two aerials depend on the relative direction of the velocity of movement of the pattern, the line joining the two aerials, and the direction of elongation of the pattern on the ground. The irregularities have been assumed to lie along the magnetic lines of force and, to a first order of approximation, to be infinitely elongated.

If the layer of irregularities is not thin, the form of the pattern will change slowly as the pattern moves across the ground, in a manner depending on the thickness. This will cause the maximum correlation of the signals at the two aerials to be less than unity. A further decrease will occur since the pattern is not infinitely elongated and the two aerials do not lie precisely along the direction of motion of the pattern. From observations of this decrease with different aerial orientations, it is in principle possible to separate these two effects and determine the thickness as well as the height of the layer of irregularities. In practice, due to the limitations of the equipment available, the results obtained even with the aerials lying almost along the direction of motion of the pattern were not accurate enough to give more than an upper limit to the thickness. The decrease in correlation also reduces the measured time-shift slightly, and a correction was applied for this.

The rate of fading of the signals was normally of the order of 5 cycles per second during periods of scintillation when measurements were made. Time-delays found over the two paths were about 0·2 and 0·4 sec. The records were normally taken for about 5 sec., and were read at intervals of 20 m.sec. Cross- and auto-correlation functions were calculated for the amplitudes of the signals received at the two aerials. Ten records were analysed, and the results are summarized in Table 1.

Table 1

Date	U.T.	Distance	Direction of irregularities observed	Height of base of *F* layer	Height of irregularities
22/6/61	22:01:03	1,169	1° W. of N.	350 km.	424 km.
22/6/61	22:02:01	1,658	6° E.	350	401
23/6/61	20:56:04	1,171	37° E.	360	416
24/6/61	21:27:02	913	34° W.	300	402
24/6/61	21:29:00	1,171	8° W.	300	396
24/6/61	21:30:06	1,545	7° E.	300	380
9/7/61	02:33:54	697	151° E.		395
10/7/61	01:22:30	788	9° E.		427
12/7/61	00:51:07	415	12° E.	260	354
12/7/61	00:52:07	292	46° E.	260	314

The distance given in column 3 refers to the distance along the ground from the observer to the point vertically below the irregularities observed. Column 5 shows the virtual height of the base of the *F* layer from an ionogram taken at Accra at the time of the observation. On all occasions on which satellite scintillations were observed, spread *F* and radio star scintillation were also present. It will be noted that the effective height of the irregularities always lies between 50 km and 100 km. above the base of the *F* layer. The value of height given above may be compared with the results of Cohen and Bowles[6], who found that the irregularities which they observe lie at or near the base of the *F* layer.

From the maximum values of the cross-correlations observed the elongation of the irregularities is found to be greater than 7 to 1, and the thickness of the scattering layer to be less than 120 km.

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A Method of Estimating Ionospheric Absorption Heights From Recovery Times

It is generally agreed that the absorption of radio waves in the ionosphere occurs at heights below about 100 km. In a particular instance it is usually possible to deduce the approximate height by indirect reasoning. For example, Hultquist[1] has used indirect evidence to place the abnormal absorption which follows intense solar flares at heights as low as 50 km. As yet there are too few direct measurements (using rockets) to provide a complete and consistent picture. This communication suggests a new method of estimating the height of the regions responsible for abnormal absorption of the kind associated with auroral disturbances.

Fig. 1 shows two examples of absorption as recorded by a riometer operating at a frequency of 30 Mc./s.

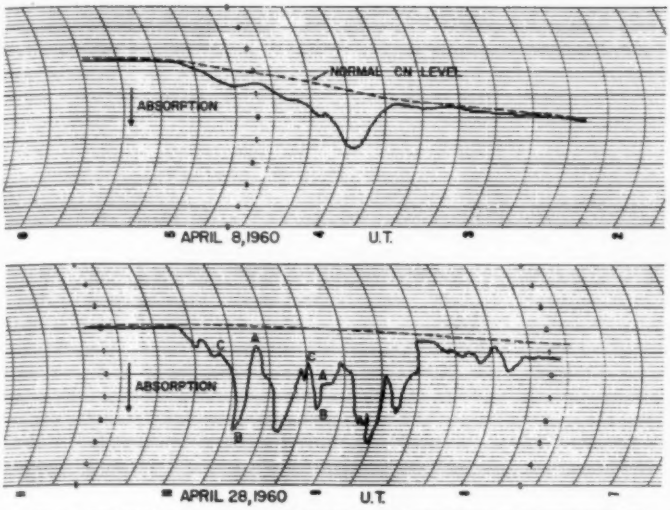

Fig. 1. Typical riometer records during abnormal disturbances. The upper trace shows a weak disturbance. The bottom trace was obtained during a severe disturbance

In each case the normal level of the cosmic noise signal in the absence of absorption is shown by the broken line. Sudden onsets of absorption (marked *AB* in Fig. 1) are frequently followed by rapid recoveries (*BC*). If it is assumed that the ionizing agency ceases to operate at the instant of peak absorption the recovery can be used for estimating the height of the region. The method depends on the fact that the absorption (expressed in decibels) suffered by a radio wave in the lower ionosphere is proportional to the number of electrons encountered (per unit area of the wave-front) in the absorbing region and is a function of the collisional frequency of the electrons.

Appleton investigated the disappearance of ionization in the lower ionosphere[2]. For small variations the ionization (electron density) should decay exponentially with a time constant:

$$T = (2\alpha N)^{-1} \text{ sec.}$$

where N is the average electron density, and α is an effective recombination or loss-rate coefficient. The quantity T is also known as the relaxation time of the ionospheric region concerned. For the present purpose it is assumed that the absorption occurs in a layer having a thickness of about one scale height which is taken to be 6·5 km. throughout the region of interest.

The electronic collisional frequencies given by Chapman and Little[3] were used to deduce electron densities required at various heights and were combined with the values of α given by Rumi[4] for day-time and night-time conditions to calculate the relaxation times (for 1 decibel of absorption) at various heights in the lower ionosphere. The relaxation times are plotted as a function of height in Fig. 2. In order to use the graphs it is necessary first to divide the observed time constant by the average absorption (expressed in decibels). Clearly, the observation would be meaningful only if the decay were measured over a change in absorption that represents only a small part of the total absorption present. There is an ambiguity in the heights deduced from Fig. 2; but this ambiguity usually can be resolved by reasoning based on the general characteristics of the regions concerned and of the absorption event. Heights deduced by the method outlined here have been compared with those determined by means of a three-frequency riometer method[5] and found to be in good agreement. I am indebted to Dr. A. Kavadas for very useful discussions and to Dr. P. A. Forsyth for valuable suggestions during the course of this investigation.

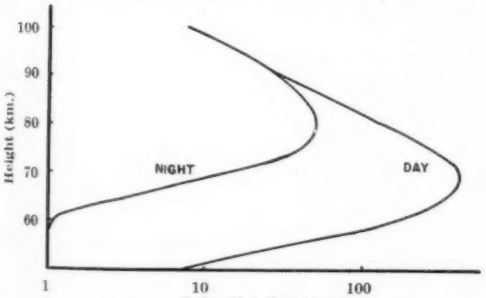

Fig. 2. Relaxation times as a function of height

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PHYSICS

Ignition of Gas by Sparks produced by breaking Wires at High Speed

INVESTIGATIONS of the conditions for ignition of inflammable gas by electrical sparking have usually been carried out with discharges produced in one of two ways: (i) by discharging a capacitor charged to a high voltage between thin fixed electrodes separated by several millimetres; (ii) by breaking low-voltage circuits at the contacts of a mechanical switch (or 'break-flash'). It has been found that less electrical energy need be dissipated in the discharge to ignite the gas if the first of these methods is adopted; for example, an 8·3 per cent mixture of methane in air can be ignited by about 0·3 mJ. by the first method, whereas an energy of about 2 mJ. is required if the second method is used.

However, recent work both in Great Britain and in the U.S.S.R. has made use of the discharge which results when a wire carrying a current is broken in gas. The first experiments in this laboratory made use of comparatively thick wires (0·022 in. and 0·012 in. in

diameter), broken so that their ends separated at about 400 cm./sec.; this is not very different from the speed of separation of electrodes of the standard break-flash[1] used in statutory testing of electrical apparatus for intrinsic safety. The currents and energies necessary to ignite gas in these conditions were only slightly smaller than those necessary with the standard break-flash. However, Berz[2] has found that, if very fine wires are broken at about the same speed, the minimum igniting currents become markedly smaller, and that they fall as the diameter of the wire is reduced. He showed that for 0·001-in. diameter tungsten wire the minimum igniting energy for an 8·3 per cent mixture of methane in air is comparable with that indicated by high-voltage experiments. He concluded that such substantial reductions in minimum igniting-energy could be obtained only when the 'electrodes' (that is, the broken wire-ends) were small compared with the critical size of the initial flame kernel in the gas (see, for example, Lintin and Wooding[3]).

It seemed likely, however, that the quenching effect of bulky electrodes could be reduced if they were rapidly removed from the kernel of the growing flame, and that the minimum igniting energies indicated by high-voltage experiments might be approached by breaking thick wires, provided that the speed of separation of the wire-ends after the break was made sufficiently high. It has been known for some time that variations in the speed of separation of electrodes of the standard break-flash apparatus (usually operated at about 300 cm./sec.) have an effect on the minimum igniting currents obtained with it, but we are not aware of previous experiments at much higher electrode speeds. Separation speeds of about 2,000 cm./sec. have now been achieved with a breaking-wire apparatus, partly based on a method developed by Gavril'chenko[4], in which a previously notched copper wire is broken by being stretched between pairs of rollers rotating at different speeds. The machine, an early model of which will be described elsewhere, differs from that of Gavril'chenko in that the length of wire stretched is comparatively long (about 35 cm.). As the wire is stretched, elastic energy is stored in it; when the wire breaks this is converted into kinetic energy and the ends of the wire separate rapidly. Although the speed of separation is substantially unaffected by the length of wire stretched, the distance over which this speed is maintained is approximately proportional to the length of wire, and in the present apparatus the separation speed of some 2,000 cm./sec. is maintained for a few millimetres.

Tests show that, although the wire is bulky (diameter 0·012 in.) compared with the wires broken by Berz, similar minimum igniting currents are obtained. Thus for a particular set of circuit conditions, a minimum igniting current of 110 m.amp. is obtained with the machine, compared with 110 m.amp. obtained by Berz for 0·005-in. steel wire, 95 m.amp. for 0·001-in. platinum wire, 82 m.amp. for 0·001-in. tungsten wire, and 185 m.amp. for the standard break-flash apparatus.

Further experiments in which notched wires 0·012 in. in diameter were broken manually (by anchoring one end and pulling the other with a pair of pliers) indicate even smaller minimum igniting currents of about 100 m.amp. for the same circuit conditions. It has not yet been possible to reproduce this manual process in a mechanized form, and further investigations are in progress.

These experiments suggest that it may be possible to design a breaking-wire apparatus which will be completely automatic and which will yield minimum igniting energies comparable with those indicated by high-voltage experiments. From a more fundamental point of view, they confirm that disturbance of the growing flame kernel by the electrodes plays a part in increasing the minimum energy necessary for ignition. If this disturbance is reduced, either by using very fine wires as in Berz's experiments, or by removing the electrodes rapidly from the flame kernel as in those reported here, energies approaching the minimum may be achieved.

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Damping without Influence on Frequency

In a recent communication[1], R. Parks and L. Maunder have directed attention to the fact that the period of oscillation of a gyrostat is not affected by damping if this were applied to both axes in a certain ratio (equal to the ratio of the moments of inertia of the uncoupled axes).

It is possible to re-formulate this condition as one of equal loss angles of the energy stores; with proper interpretation the same interesting and potentially useful result holds for ordinary (non-gyroscopic) systems and it can be extended to systems with more than one degree of freedom.

For a system with one degree of freedom the assertion will appear as an obvious conclusion from the Parks–Maunder result if we follow the approach of ref. 2, for there it is shown how the system considered by these authors can be transformed into a dynamically equivalent system consisting of a mass and a spring which are both subject to damping. The argument is based on the fact that, 'seen' across a gyrostatic coupler, a mechanical impedance Z looks like an admittance:

$$Y = Z/C^2n^2 \qquad (1)$$

where Cn is the moment of momentum of the gyrostat rotor around its axis of rotation. From this it follows that the mass impedance Bp attached to axis 2 of the gyrostat appears from axis 1 as the admittance $D_2p = Bp/C^2n^2$ of a spring; the viscous damper of constant c_2, which is linked co-resistively[3] to the mass, transforms into a viscous damper of constant $K_2 = C^2n^2/c_2$, which is linked co-yieldingly[3] to this spring. As the combined impedance of the uncoupled axis 2 amounts to $Bp + c_2$, we need not in fact even evaluate these individual components but can write directly from equation 1 the value of the impedance which the coupler transfers to axis 1 as $C^2n^2/(Bp + c_2)$. The governing equation for axis 1 then reads:

$$Ap + c_1 + \frac{C^2n^2}{Bp + c_2} = 0 \qquad (2)$$

This is, of course, equivalent to the equation derived in ref. 1 by purely algebraic manipulation.

To prove the general assertion, assume first that we deal either with a non-gyroscopic system consisting

of masses and springs only, or with a gyroscopic system which, according to ref. 2, can be transformed into an equivalent system of this kind. Let $p_i = j\omega_i$ be one of the n frequencies for which the governing equations for this loss-less system are identically fulfilled. The value ω_i enters these equations only via the expressions for the mass impedances $Z_{mih} = p_i m_h = j\omega_i m_h$ or the spring admittances $Y_{sih} = p_i D_h = j\omega_i D_h$, or via the reciprocals.

The corresponding expressions for the damped system are:

$$Z'_{mih} = p_i m_h + K_h = p_i m_h + \alpha m_h, \text{ say, and}$$
$$Y'_{sih} = p_i D_h + x_h = p_i D_h + \alpha D_h \qquad (3)$$

for the conditions considered, where α is a constant, usually positive. It will be seen that for values $p'_i = j\omega_i - \alpha$, we get:

$$Z'_{mih} = (j\omega_i - \alpha)m_h + \alpha m_h = Z_{mih}$$
$$Y'_{sih} = (j\omega_i - \alpha)D_h + \alpha D_h = Y_{sih} \qquad (4)$$

This means that for this particular value of the complex frequency the coefficients of the governing equation(s) for the damped system are the same as for the undamped system. These equations are therefore solved by the same set of complex phasors as before, and the new case differs from the old one merely in so far that these phasors now describe a damped oscillatory motion of the form u_l = real part of $U_l . \exp(j\omega t) . \exp(-\alpha t)$ instead of $U_l.\exp(-j\omega t)$.

If it is not possible or not desirable to transform a gyroscopic system into an equivalent non-gyroscopic system we have to consider the contributions arising from the gyroscopic coupling terms, that is, in the present notation from terms due to asymmetrical mutual coupling impedances of the form $Z_{gl} = \pm C^2 n^2$. These impedances do not change when damping is added to the system nor—being independent of frequency—are they influenced by the change from p_i to p_i'. Hence the conclusion reached from equations (4) is not disturbed and the assertion remains valid.

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Cryoscopic Effect of Separation and Concentration of Solutes

The normal method of concentrating a substance in solution is by distillation. In the course of certain experiments it was found that it is possible to do so to a certain extent by refrigeration.

A column of skim milk in a 250-ml. cylinder was frozen between $-5°$ and $-10°$ C. The frozen column was taken out and allowed to thaw at room temperature (24°–27° C.) in about 2·5 hr. The melted column showed a graded separation, ranging from solid concentration at the bottom to a thin, watery layer at the top. Portions of 10 ml. were carefully collected from the top and bottom of the column with the least possible disturbance, and analysed for total solids and milk sugar.

Table 1. Concentration of Skim Milk by Cryoscopy (gm./100 ml.)

Portion	Total solids	Lactose
Top	3·6	2·23
Bottom	30·8	7·31
Milk before freezing	8·1	4·63

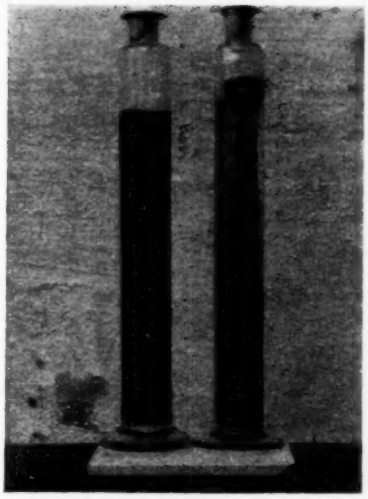

Fig. 1. Columns of potassium permanganate. Left, solution before freezing; right, after freezing. Note the cone of concentrated solute in the frozen column

These figures clearly bring out the high concentration of the solids at the bottom of the column on freezing. It is also noteworthy that the degree of concentration differs from constituent to constituent.

Use of whole milk interferes only with the visible separation of the solids, since the watery top-layers are masked by a plug of light fat.

Table 2. Concentration of Whole Milk by Cryoscopy (gm./100 ml.)

Portion	Total solids	Fat	Solids-not-fat
Top	8·439	3·24	5·199
Bottom	30·66	7·45	23·21
Milk before freezing	14·03	5·2	8·83

The 'cryoscopic effect', as the phenomenon may be called, is common to crystalloids as well. Solutions of copper sulphate, potassium permanganate and potassium ferrocyanide, similarly frozen and thawed, yield a higher concentration of the salts at the bottom of the column, at once evident by the greater intensity of colour. The disparity in concentrations of the salts between the top and bottom layers of the melted column can be seen in Table 3.

Table 3. Concentrations of Crystalloids by Cryoscopy (gm./100 ml.)

Portion	Copper sulphate	Potassium permanganate	Potassium ferrocyanide
Top	2·328	0·035	0·382
Bottom	7·950	0·230	6·410
Solution before freezing	2·739	0·052	1·008

The frozen column of potassium permanganate exhibits a vivid concentration gradient of the salt in the form of a purple cone with its base at the bottom. It is perhaps typical of cryoscopic concentration of solutes. Possibly this can be verified by using X-rays or by the tracer technique in relatively opaque frozen columns of other solutes.

In order to obtain a three-dimensional picture of the concentration of solutes in the frozen column, annular portions of the melts (simultaneously with analyses of top and bottom portions in another column) were analysed, as the thawing progressed.

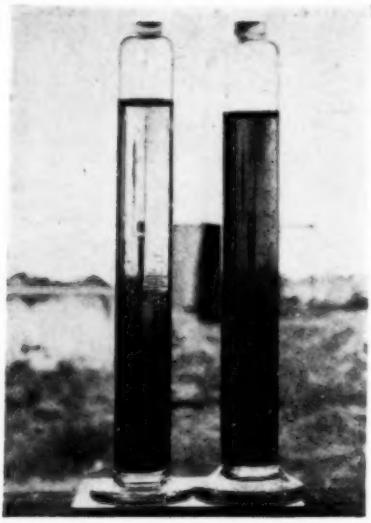

Fig. 2. Columns of potassium permanganate. Left, after freezing and thawing. Note heavy concentration of solute at the bottom. Right, original solution without freezing. Dark streaks in the centre of the columns are due to photographic effects

The average concentrations of salt in the concentric layers of the column from outside inwards, as well as the concentrations of the top and bottom in the second column of each salt, are given in Table 4.

Table 4. Concentration of Annular Layers and Vertical Portions of Frozen Columns of Solutions (gm./100 ml.)

Portion	Copper sulphate	Potassium permanganate	Potassium ferrocyanide	Skim milk
Outer layer	4·05	0·091	2·192	11·49
Thawing time (min.)	30	35	28	40
Middle layer	2·775	0·08	—	8·962
Thawing time	60	75	—	85
Inner layer	1·495	0·06	0·840	5·814
Thawing time	135	110	65	145
Top and bottom portions in second column				
Top	2·328	0·035	0·382	2·378
Bottom	7·95	0·23	6·410	26·918
Solution before freezing	2·739	0·052	1·008	9·2834

These results give an insight into the pattern of concentration of solutes on freezing. The heaviest concentration seems to be a hollow ring at the bottom of the column. It is probable that a predictable relation exists between the solute concentration at the bottom of the frozen column and the concentration of the original solution, the molecular weight of the substance, the height and diameter of the frozen column.

The cryoscopic phenomenon may be of general use in analytical procedures and manufacturing processes, particularly where dilute solutions are involved and distillation is unsuitable. It may be possible to concentrate the heavy water fraction in water by repetitive cryoscopy for the atomic energy industry.

I thank Mr. A. E. Owen for his assistance in the analyses of the samples, and Drs. Anantakrishnan and Sethu Rao for experimental facilities at the National Dairy Research Institute, Bangalore.

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CHEMISTRY

Differential Thermogravimetry versus Differential Thermometry in studying the Pyrolysis of Polymers

Ordinary chemical methods of analysis are impractical for studying the pyrolysis of highly cross-linked polymers, because such materials are extremely infusible, insoluble and non-volatile. Techniques such as differential thermometry and differential thermogravimetry are therefore being used more and more for this purpose. Differential thermometry measures the net heats of reaction produced by chemical change as a function of temperature, whereas differential thermogravimetry measures loss of weight. Since the endothermic breaking of and the exothermic forming of bonds occur simultaneously in the pyrolysis of substances, differential thermometric information can be misleading. For example, if, at a given temperature, the heats of the endothermic reactions were exactly equal to the heats of the exothermic ones, a differential thermometric peak would be absent. This would lead to the false conclusion that no thermal decomposition had occurred. Changes in specific heat and in thermal conductivity of a polymer during pyrolysis also complicate the differential thermometric picture, especially when quantitative information is desired. Since differential thermogravimetry measures losses of weight during pyrolysis rather than heat changes, it is not subject to the above-mentioned complications, and should therefore give a more reliable quantitative estimate of the relative thermal stabilities of different polymers.

An example of this difference in reliability of the two methods may be had by comparing the corresponding data obtained for two epoxide polymers having different chemical structures. These polymers were prepared by treating dipentene dioxide in one case and vinyl*cyclo*hexene dioxide in the other case with one mole of methyl nadic anhydride per epoxy equivalent, using 1 per cent N,N-dimethylbenzylamine as a catalyst.

Both the differential thermometric and differential thermogravimetric experiments were performed at a heating rate of 5 deg. C./min., the former in nitrogen at atmospheric pressure and the latter *in vacuo*. Descriptions of the apparatus and procedures have been given previously[1,2]. The differential thermogravimetric curves in Fig. 1 clearly show that the vinyl*cyclo*hexene dioxide polymer is more thermally stable than the dipentene dioxide one. The former polymer shows a maximum rate of loss of weight at 375° C., whereas the latter one shows a maximum rate at 265° C. This large difference in thermal stability of the two polymers is not revealed by the differential thermometric curves in Fig. 2. Actually, the differential thermometric peaks at approximately 275° C. for each of the two polymers indicate that the vinyl*cyclo*hexene dioxide polymer has its maximum rate of decomposition at the same temperature as the dipentene dioxide polymer. Furthermore, if the two methods are compared for only one polymer, the differential thermometric data can still be unreliable. For example, the differential thermometric curve for vinyl*cyclo*hexene dioxide indicates that much more decomposition occurred at 275° C. than at 360° C. This indication is also contrary to the more reliable differential thermogravimetry data.

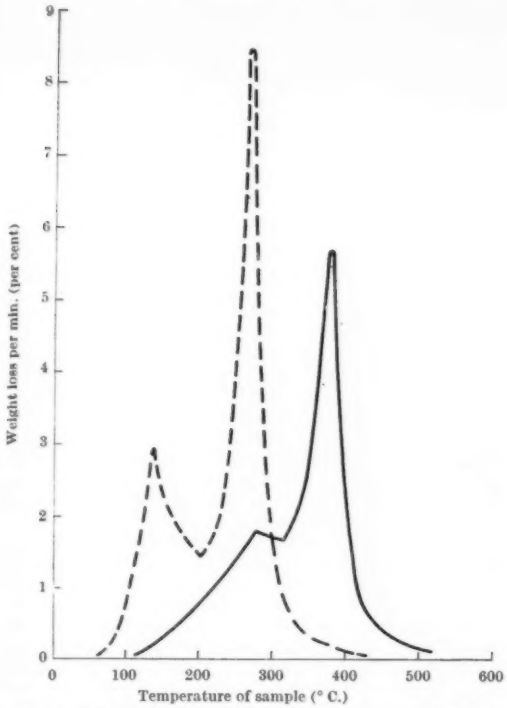

Fig. 1. Differential thermogravimetric curves. – – –, Dipentene dioxide polymer; ——, vinylcyclohexene dioxide polymer

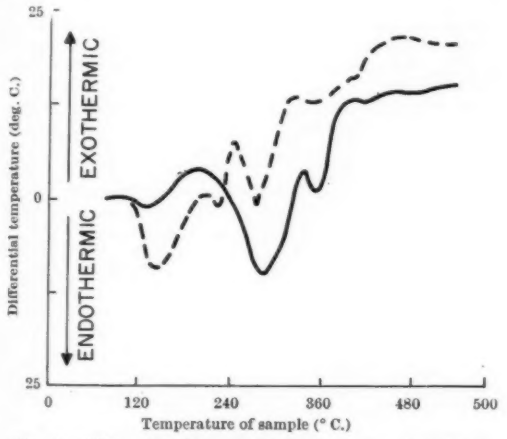

Fig. 2. Differential thermometric curves. – – –, Dipentene dioxide polymer (3·3 gm.); ——, vinylcyclohexene dioxide polymer (1·6 gm.)

Despite these shortcomings of differential thermometry as a tool for studying the pyrolysis of polymers, it can sometimes be very useful in revealing, or in clarifying, certain mechanisms of thermal degradation. For example, it has been shown by differential thermometry that isomerization of residual epoxy groups in certain epoxide polymers plays an important part in the decomposition mechanism[2]. It is therefore recommended that differential thermogravimetry be used to obtain quantitative information on the pyrolysis of polymers, especially highly cross-linked ones, and that differential thermometry be used as a qualitative adjunct.

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Ionization Potentials of *cis* and *trans* Dichloro- and Dibromo-ethylenes

THE ionization potentials of pure *cis* and *trans* dichloro- and dibromo-ethylenes have been obtained by electron impact and by photon impact methods[1]. Table 1 gives my results and those obtained by Walsh[2] from Rydberg series.

Table 1. IONIZATION POTENTIALS (IN EV.) OF *cis* AND *trans* DICHLORO- AND DIBROMO-ETHYLENES

Substances	Walsh's results	Electron impact method	Photon impact method
cis $C_2H_2Cl_2$	9·66	9·67 ± 0·05	9·66 ± 0·02
trans $C_2H_2Cl_2$	9·96	10·00 ± 0·05	9·64 ± 0·02
cis $C_2H_2Br_2$		9·69 ± 0·05	9·45 ± 0·01
trans $C_2H_2Br_2$		9·54 ± 0·05	9·46 ± 0·01

I have observed in the photon impact efficiency curves the presence of singular points which I believe correspond to vibrationally excited states of the molecular ion. An example of this is given here for *trans* dichloroethylene (Fig. 1).

The singular points are as follows: *cis* dichloroethylene (9·77 ± 0·02 eV.); *trans* dichloroethylene (9·81 ± 0·02; 9·97 ± 0·03 eV); *cis* dibromoethylene (9·62 ± 0·02; 9·79 ± 0·02 eV.); for the *trans* dibromoethylene no sharp point appears.

From the values of the ionization potentials of ethylene, methyl chloride, and ethyl chloride, which are respectively 10·51, 11·28 and 10·97 eV. (ref. 3), it must be concluded that the electron

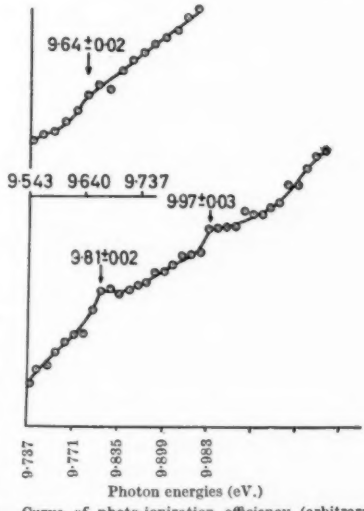

Fig. 1. Curve of photo-ionization efficiency (arbitrary scale) against photon energies (electron volts) for *trans* dichloroethylene

removed in the case of the dihalogenoethylenes is a $\pi C{=}C$ electron. This and the order of magnitude of the jumps between two consecutive singular points indicate that the vibration involved is probably the C=C stretching vibration.

The electron impact and photon impact values are in rather good agreement for *cis* dichloro- and *trans* dibromo-ethylene, but an important discrepancy exists for *trans* dichloro- and *cis* dibromo-ethylene. From the vibrational separations of about 0·17 eV. observed for both substances, we conclude that the electron impact data correspond to the appearance of vibrationally excited states of the ion ($v=2$ for *trans* dichloroethylene and $v=1$ for *cis* dibromoethylene) and that the two other ions appear in the $v=0$ vibrational level for both photon and electron ionization.

Discrepancies of this nature between photon impact and electron impact ionization potentials are quite frequent and the interpretation given here seems to be of great generality. It conforms to the ideas of Mulliken[4], who considers photon impact potentials as "adiabatic" and electron impact potentials as "vertical" ones.

Further, from the equality of the formation enthalpies of the ions[1], it may be assumed that only one equilibrium configuration of the nuclei exists for the ion coming from a *cis* or from a *trans* isomer. This is in agreement with the theoretical work of Mulliken[5] on the $C_2H_4^+$ ion, the configuration of which he considers to be a crossed one.

By treating the dichloroethylene molecule and ion as diatomic vibrators with respect to the C=C bond, it may be shown that the results given here can be interpreted by assuming that the distance C=C is 0·035 Å. larger in the *cis* molecule and 0·065 Å. larger in its ion than in the corresponding *trans* dichloroethylene states.

I am indebted to the British Council and to the Patrimoine of the University of Liège for a grant, and to Prof. W. C. Price for permitting me to carry out the photo-ionization measurements in his laboratory at King's College, London. I wish to thank Prof. L. D'Or for the active interest he has shown in this work.

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Rapid Determination of Ytterbium in Aqueous Solutions and in Thulium Oxide by Neutron Activation

It is known that when natural ytterbium is irradiated with neutrons, ytterbium isotope of 6 sec. half-life is formed[1], and that this emits γ-rays of 0·104 MeV. and of 0·212 MeV.

In the present work, a sample (0·07 ~ 0·3 gm.) is placed in the nuclear reactor *JRR*-1 for 6 sec. to be irradiated with neutrons at a flux of 3×10^{11} neutrons per cm.²/sec. and then the decay of the mixture of activities produced is determined by use of a 2-in. thick, well-type sodium iodide crystal attached to a 256-channel pulse-height analyser. In each of successive γ-spectra obtained, the height of the photopeak of the 0·104-MeV. γ-ray from 6 sec. ytterbium isotope is measured, and the decay curve is made by plotting the log peak height against time. Identification was by the γ-energy and the half-life. A calibration curve was prepared by activating and counting ytterbium solutions containing known amounts ranging from 0·01 to 1 mgm. of ytterbium.

Table 1. Results of Analyses of Thulium Oxide

Sample	Sample weight used (mgm.)	Ytterbium found (per cent)	Dysprosium found (p.p.m.)
I	122	0·057	5·6
	76	0·062	6·2
II	124	0·083	5·5
	70	0·075	6·0

In the analyses of an aqueous sample, the coefficient of variation of single analyses was 5 per cent, where the content of ytterbium was 0·064 per cent and eight aliquots was used each of which was 0·25 ml. in volume. Since interfering activities were practically negligible and activities produced were on a level very suitable to the counter used, this value is felt to be the approximate lower limit of the coefficient of variation in this method.

In thulium oxide samples the resolution of the decay curve showed two components : a 6-sec. and a 1·3-min. The latter was identified to be dysprosium-165*m*. Dysprosium was then determined quantitatively by use of a calibration curve made by the method similar to that for the case of ytterbium mentioned above. Results of analyses of thulium oxide samples as fine powder are shown in Table 1.

Since ytterbium found and dysprosium found in Table 1 are nearly independent of sample weight used, it is deduced that self-shielding for neutrons in these samples was negligible. It has also been deduced from decay curves that, when a thulium oxide powder contains 6 p.p.m. dysprosium, the lower limit of the detection of ytterbium is about 0·01 per cent.

By a similar method using a 0·212-MeV. photopeak instead of a 0·104-MeV. photopeak reliable analyses of decay curves could not be attained.

I am indebted to Prof. Shoji Makishima for helpful suggestions, to Dr. Toshio Nakai for permission to use laboratory facilities, and to Dr. Yuichiro Kamemoto for help in the γ-ray spectrometry.

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Stability Constant of Copper(II) Complexes of Reduction Products of 2,2′-Dipyridyl

The ligand 2,2′-dipyridyl (DP), its derivatives and analogues form an extremely interesting and important class of ligands. Their complexes possess some particular properties, presumably as a consequence of π-electron system of the ligand. To elucidate the role of the π-electron system, the study of complex-forming properties of the reduction product of 2,2′-dipyridyl was undertaken. Although

we are at the very beginning of a systematic investigation, nevertheless, we achieved some results worthy of publication.

2,2′-Dipiperidyl (DPP) was prepared from DP by hydrogenation at 100° C. under 120 atmospheres pressure, using Raney-nickel catalyst. The yield exceeds 90 per cent. The boiling-point of DPP at 6 mm. of mercury pressure is 100–105° C. 2,2′-Pyridylpiperidyl (PPP) was prepared also from DP by tin-hydrochloric acid reduction; after the reduction PPP was extracted from the alkalized solution by chloroform. The yield is about 70 per cent, the boiling point 265–266° C.

The acidic dissociation constants were determined by potentiometric titrations: 10^{-2} mole/l. solutions of ligands were titrated with 1 mole/l. hydrochloric acid at 20° C.; the solutions were of 0·1 mole/l. for potassium chloride. The values of acidic dissociation constants are shown in Table 1. For comparison acidic dissociation constants of DP (ref. 1) are also given.

Table 1

	K_1	K_2	K_1K_2	K_1/K_2
DP	1·59	$4{\cdot}27 \times 10^{-5}$	$6{\cdot}77 \times 10^{-5}$	$3{\cdot}72 \times 10^{4}$
PPP	10^{-2}	$7{\cdot}95 \times 10^{-10}$	$7{\cdot}94 \times 10^{-12}$	$1{\cdot}26 \times 10^{7}$
DPP	10^{-7}	$6{\cdot}90 \times 10^{-11}$	$6{\cdot}90 \times 10^{-18}$	$1{\cdot}45 \times 10^{4}$

As can be seen, the basicity of nitrogens increases with hydrogenation. It is interesting that the ratio K_1/K_2 is nearly the same in the case of DP and DPP, while it differs strongly from PPP due to the inequality of nitrogens in this compound.

The stability constants of copper(II) complexes of DPP and PPP were determined also by potentiometric titrations under the same conditions as the acidic dissociation constants of ligands. The values are tabulated in Table 2.

Table 2

	K_1	K_2	K_1K_2
PPP			$1{\cdot}59 \times 10^{12}$
DPP	$3{\cdot}16 \times 10^{10}$	$2{\cdot}75 \times 10^{7}$	$8{\cdot}69 \times 10^{17}$

It is worth mentioning that the ratio K_1/K_2 is exactly the same as in the case of *N*,*N*′-dimethylethylenediamine copper(II) complex studied by Irving and Griffiths[2].

In the case of PPP the formation function does not show a step at $\bar{n} = 1$. So the product, K_1K_2 could only be calculated.

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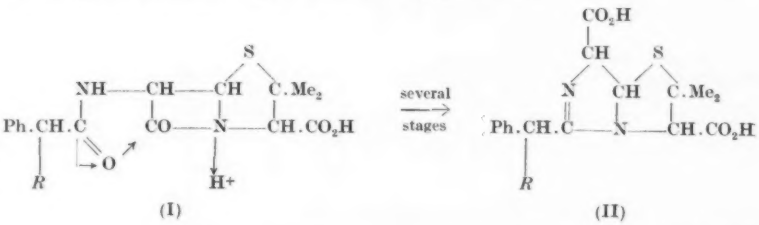
Some Novel Acid-stable Penicillins

Rapid inactivation of penicillins *F*, *G*, *K* and *X* in acid solution was demonstrated at an early date[1], and was widely assumed to be a general property until 1954, when phenoxymethylpenicillin (penicillin *V*) was shown to be relatively stable towards mineral acids[2]. The recent isolation in these Laboratories of 6-aminopenicillanic acid[3] permitted the synthesis of many hitherto inaccessible penicillins, including an extended series of acid-stable α-aryloxyalkylpenicillins[4] of which one, α-phenoxyethylpenicillin, is now in clinical use. Both penicillin *V* and its newer homologues are well absorbed from the gastrointestinal tract and hence can be given by mouth, but they show useful antibacterial activity only against Gram-positive bacteria. Benzylpenicillin (penicillin *G*) has a broader antibacterial spectrum, but is not suitable for oral administration. We therefore prepared a series of α-substituted benzyl penicillins (I) in the hope of finding an acid-stable penicillin active against Gram-negative as well as Gram-positive bacteria.

The α-methoxy-, α-chloro-, and α-benzyloxycarbonylamino-compounds were prepared by treating 6-aminopenicillanic acid in aqueous sodium bicarbonate with an acetone solution of either the chloride or the mixed ethoxyformic anhydride of the appropriate α-substituted phenylacetic acid. The resulting penicillins were extracted from the acidified reaction mixture into ether, then re-extracted into aqueous sodium bicarbonate and freed from water by evaporation at low temperature and pressure. Hydrogenation of an aqueous solution of the sodium salt of α-benzyloxycarbonylaminobenzylpenicillin (I; $R = NH.CO_2.CH_2Ph$) over a palladium catalyst gave α-aminobenzylpenicillin[5].



Specimens (50 mgm.) of each of the penicillins (I; R = H, OMe, Cl, and NH_2) were added to a mixture of 0·2 *N* aqueous hydrochloric acid (25 ml.) and ethanol (25 ml.) maintained at 35° C. and aliquot portions of each solution were withdrawn at intervals and the penicillin content determined by colorimetric assay with hydroxylamine[6]. The half-life of each penicillin was then read from the straight-line plot of log concentration against time. The results shown in Table 1 indicate that α-methoxy-, α-chloro-, and α-amino-benzylpenicillin are all much more stable to acid than is benzyl penicillin itself, while the chloro- and amino-compounds are also more stable than penicillin *V* (half-life 160 min.).

Table 1

	R = H	R = OMe	R = Cl	$R = NH_3^+$
Half-life of penicillin (I) in 50 per cent aq. EtOH at *p*H 1·3 and 35° C.	3·5 min.	77 min.	300 min.	660 min.
pKa of side-chain acid, Ph.CH.COH, R in 50 per cent aq. EtOH	5·6	4·6	4·0	3·2

Evidently benzylpenicillin can be rendered more stable to acids by the introduction into the α-position of the side-chain of electron-attracting substituents which might be expected to hinder the electron displacements initiating rearrangement to the isomeric

biologically inactive penillic acid (II)[7]. Such stability would be expected to vary with the inductive effect of the substituent and hence to be roughly proportional to the strength of the side-chain acid from which the penicillin is derived. Support for this idea was obtained by potentiometric titration of the appropriate α-substituted phenylacetic acids against standard alkali in 50 per cent aqueous ethanol. Correlation of the *pKa* values calculated by the half-neutralization method with the acid half-lives of the corresponding penicillins is shown in Table 1.

As well as being the best of the new penicillins from the point of view of stability to acid, α-aminobenzylpenicillin also showed useful activity against a wide range of Gram-positive and Gram-negative bacteria both *in vitro*[8] and *in vivo*[9] and was well absorbed from the gastro-intestinal tract[9]. The epimer derived from D(−)α-aminophenylacetic acid was more active against most organisms than that from the L(+)-acid. Details of the preparation of it and some of its analogues will be published elsewhere.

We thank Dr. G. N. Rolinson *et al.* and Mr. D. M. Brown *et al.* for the bacteriological and chemotherapeutic testing of the penicillins and Mr. D. Neal for experimental assistance.

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Crystallization of Isotactic Polypropylene using a Light Depolarization Technique

The intensity of depolarized light transmitted by a thin film of isotactic polypropylene, crystallizing isothermally on a polarizing microscope hot stage was followed using a photomultiplier unit with an appropriate recorder system[1,2]. For the slower rates of crystallization, the cathode ray oscillograph–camera arrangement was replaced by a Honeywell Brown m.V. recorder. All measurements were made within the linear operating range of the equipment.

Dried sections (150–200μ) of unpigmented polymer chip of intrinsic viscosity 2·7 dl./gm., measured in tetralin at 135° C., were mounted between cover slips using *MS* 550 silicone oil as an immersion medium to reduce oxidation of the sample during melting. Specimens were melted at 270 ± 3° C. for 30 min. followed by rapid transfer (< 1 sec.) to the microscope hot stage maintained to within 0·5° C. of the desired crystallization temperature. Typical

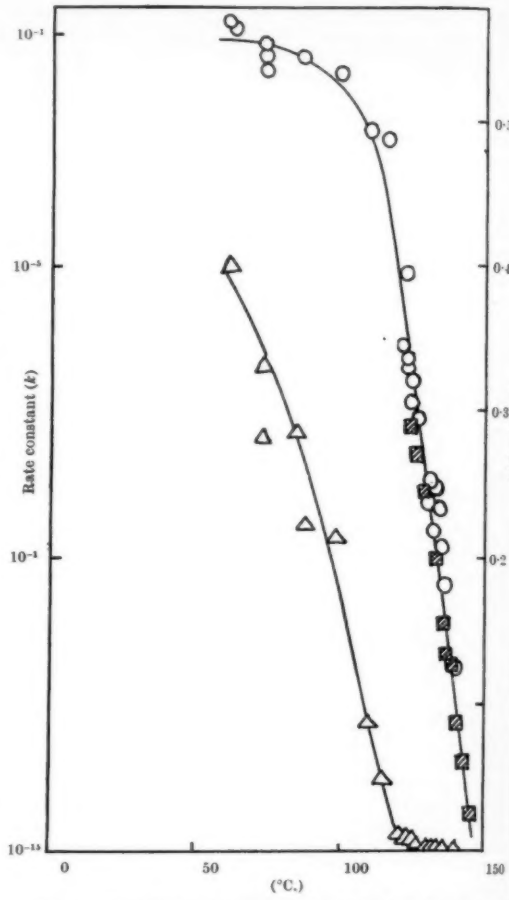

Fig. 1. Crystallization parameters for isotactic polypropylene polymer. Triangles, reciprocal of the half-times of crystallization temperatures (this work); circles, rate constants versus crystallization temperatures (this work); below and at 115° C. *K* is expressed in sec.$^{-2}$; above 115° C. *K* is expressed in sec.$^{-3}$

sigmoidal curves of the change in photomultiplier output with time were obtained and used as a measure of the extent of the crystallization. Results were fitted to Avrami equations after expressing them as a proportional change $(I_c - I_t)$ relative to the overall primary change $(I_c - I_0)$ associated with any isotherm-al[2]. I_c, I_t and I_0 are respectively equal to the final, intermediate and initial photomultiplier outputs. For the lower crystallization temperatures (< c. 80° C.) secondary or 'after' crystallization behaviour is frequently pronounced.

A change in the mechanism of nucleation occurs in the region of 115° C. Above this crystallization temperature spherulitic growth occurs from predetermined or simultaneous nuclei, while below 115° C. the structures formed are granular, and birefringent and nucleation is sporadic. The rate constants calculated from light depolarization isothermals (Fig. 1) agree well with published values derived from dilatometric measurements[3] for an isotactic polypropylene of similar intrinsic viscosity. The rate measurements presented here establish the utility of the technique for studying crystalliza-

tion behaviour in polymers, and also its applicability over a wider temperature-range than most other established methods. Strain birefringence limits the use of the method at large degrees of supercooling where poor heat transfer is also significant[2].

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BIOCHEMISTRY

Synthesis of Succinate from Acetate

In a previous communication[1], evidence was presented that an enzyme system, isolated from pig heart, catalysed the oxidation of acetate to succinate. Aconitase, assayed by the method of Racker[2], was not detectable and the addition of malate to the incubation mixture did not increase the incorporation of acetate into succinate. Consequently, the formation of succinate was attributed to a Thunberg condensation and the slow rate (7 mμmoles/br./mgm. protein) was explained by the unfavourable equilibrium[3].

This explanation has been tested by attempting to measure the formation of acetate-^{14}C from succinate-2.3-^{14}C with preparations capable of incorporating acetate-^{14}C into succinate. Such experiments failed to demonstrate any significant formation of acetate, acetyl co-enzyme A or acetyl phosphate (when a preparation of transacetylase was added to the incubation mixture). These results indicate the absence of the Thunberg reaction and suggest that succinate is formed via the established reactions of the Krebs cycle—the slow rate being due to the extremely low activity of aconitase. Support for this view comes from the finding (Dr. T. M. Sutherland, private communication) that the incorporation of acetate into succinate disappears after dialysis of the enzyme system, but is restored by the addition of oxaloacetate.

It thus appears that the interpretation of the data in terms of a Thunberg condensation, for which I was solely responsible, was erroneous.

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Conversion of Fibrin to Desmofibrin

In experiments on fibrin stabilization great difficulty has been encountered in obtaining a substrate free from fibrin-stabilizing factor (FSF)[1–5].

Such a substrate can be obtained from a fibrinogen preparation made by various methods, for example, from Kekwick's preparation[6] or, from oxalated plasma clotted with citrate thrombin[7]. From a clot mechanically released from a solvent or serum, a 2–4 per cent fibrin solution in urea solution at *p*H 5·2 (20 gm. urea in 100 ml. acetic buffer adjusted to *p*H 5·2 with acetic acid) is prepared.

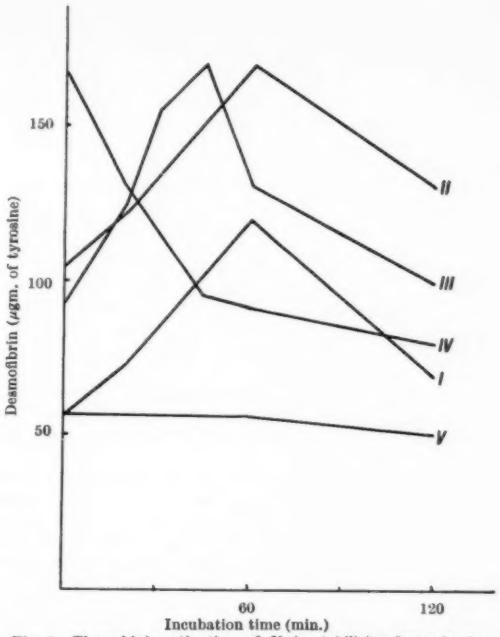

Fig. 1. Thrombinic activation of fibrin stabilizing factor in the presence of calcium. Incubated mixture: thrombin + FSF + calcium chloride. Curves I, II, V, 0·05 unit thrombin/ml.; curve III, 0·1 unit thrombin/ml.; curve IV, 1·0 unit thrombin/ml.; curves I, III, IV, V, 20 min.; curve II, 45 min. reaction of fibrin with incubated mixture; curve V, incubated mixture: thrombin + FSF without calcium chloride (calcium chloride was added just before mixing with fibrin monomer)

A fibrin solution from which the precipitates, regarded as being denatured, had been removed by centrifugation, was diluted 25–50 times in Palitzsch buffer solution[8] at *p*H 7·6, in which a fibrin network was immediately formed. This procedure of dissolving and repolymerizing of fibrin was repeated ten times.

Traces of thrombin could be identified in the diluent after the third repolymerization and removal of the clot. In subsequent operations, it was not possible to demonstrate the presence of thrombin, though it is known to be absorbed on the fibrin[9].

The product finally obtained is considered to be a fibrin monomer and it possesses the following properties: it clots immediately after dilution and neutralization, the time of polymerization depends on the concentration of salt and is lengthened if distilled water is used for dilution, the addition of thrombin has no effect on polymerization-time, provided that the concentrations of salt are constant.

The clot, that is, the fibrin polymer obtained from the monomer described here, is soluble in urea solution and in mono-chloracetic acid even when thrombin and calcium are added.

Stabilized fibrin polymer or desmofibrin is obtained by adding FSF, thrombin and calcium.

The fibrin polymer stabilization time and the amount of desmofibrin obtained in a definite period of time (for example, 20 min.) are proportional to the amounts of FSF added. With a constant FSF fibrin polymer, stabilization time and the amount of desmofibrin formed depend on the amount of thrombin added.

The experiment shown in Fig. 1 was carried out in two stages. In the first stage, three test-tubes con-

taining a buffer solution at *p*H 7·6, FSF derived from platelets[10], and calcium in equal concentrations with citrate thrombin[7], the activity of which was, in the first test-tube 0·05 unit/ml., in the second, 0·1 unit/ml., and in the third 1 unit/ml. of the mixture, were incubated. At various intervals during incubation the incubated mixture was added to prepared test-tubes containing fibrin monomer.

In the second stage at various intervals after mixing the fibrin monomer with the incubated mixture the reaction was interrupted by adding mono-chloracetic acid and the amount of desmofibrin contained in the tubes was measured[11].

The experiment shows that during the reaction with FSF, when calcium and thrombin 0·05 unit/ml. and 0·1 unit/ml. are present, the fibrin-stabilizing activity increases and, during further incubation, it decreases (curves I, II, III).

Where 1 unit/ml. of thrombin is present, the high fibrin-stabilizing activity of the incubated thrombin, FSF and calcium is observed at the beginning of incubation (curve IV).

On comparing curves I and II, it will be seen that where 0·05 unit/ml. of thrombin is present, the peak of fibrin-stabilizing activity is observed 60 min. after incubation of thrombin, calcium and FSF. The conversion of fibrin polymer into desmofibrin takes place gradually, since after a 20-min. reaction of the incubated mixture with the fibrin polymer (curve I) it contains 120γ, after 45 min. (curve II) fibrin polymer contains 170γ of tyrosine expressed as the equivalent of desmofibrin.

During the incubation of thrombin and FSF without calcium, the fibrin-stabilizing activity does not increase (curve V). Stabilization of the fibrin polymer with this mixture and the addition of calcium correspond at the onset of incubation to that of the control in which all elements required in order to obtain desmofibrin are mixed simultaneously and, after further incubation, the stabilizing activity of the incubated mixture is lower than that of the control.

The experiment indicates that the stabilized fibrin, that is, desmofibrin, is a product of two-step reaction: in the first step thrombin acting on fibrinogen, not necessarily in the presence of calcium, produces the fibrin and by acting on FSF in presence of calcium thrombin, produces an active fibrin-stabilizing factor.

In the second step, during reaction of fibrin with active fibrin-stabilizing factor, desmofibrin is formed.

The problem of the significance of calcium during the reaction of fibrin polymer with active fibrin-stabilizing factor has not yet been elucidated. Active fibrin-stabilizing factor created during the time of reaction with thrombin in the presence of calcium, after addition of sodium citrate, results in desmofibrin during the reaction with fibrin polymer.

With the same conditions, but with potassium oxalate added to the active fibrin-stabilizing factor, desmofibrin is not produced in the reaction with fibrin polymer.

The two-step formation of desmofibrin is as follows:

First step: (*a*) fibrinogen + thrombin → fibrin

(*b*) FSF + $\xrightarrow[Ca^{++}]{\text{thrombin}}$ active fibrin-stabilizing factor

Second step:

fibrin + $\xrightarrow[Ca^{++}\ (?)]{\text{active fibrin-stabilizing factor}}$ desmofibrin

The fact that the formula given above differs from other known findings[12] can be explained as being the result of using fibrinogen preparations containing FSF instead of the sol of fibrin monomer free from fibrin-stabilizing factor, for work on fibrin-stabilization.

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Catalysis of Incorporation of ^{14}C-Uridine into Rat Lens Ribonucleic Acid by Rat Lens Enzyme System

Mammalian lenses may now be considered to be centres of the active metabolism of protein on the basis of the recent findings of Devi, Lerman and Sarkar[1] on the existence in rat lens of a very active system which catalyses the incorporation of amino-acids into lens ribonucleic acid (RNA) and proteins, and on the basis of the occurrence of many proteases in rat lens as demonstrated by Zeller and Devi[2] and Devi[3,4]. The relatively high concentration of RNA in rat lens as reported by Dische and Devi[5] and its key role in protein synthesis as shown by various investigators[6,7] has stimulated us to examine whether or not there also exists in rat lens any system capable of synthesizing RNA.

Rats 6–8 weeks old and weighing approximately 100–120 gm. each were given intraperitoneal injections of 0·2 ml. of 2-^{14}C-uridine (specific activity 2·2 × 10^6 c.p.m.) prepared in normal saline. The animals were killed at different time-intervals after injection. The lenses from each rat were immediately removed and homogenized in 1 ml. of 0·25 *M* sucrose in the cold and to the latter 4 ml. of 5 per cent cold trichloracetic acid (TCA) were added. The TCA-insoluble material was afterwards washed three times with cold 5 per cent TCA, once with alcohol, once with methanol/chloroform/ether (2 : 1 : 1 proportion) mixture at 50° C. and then twice with ether. The TCA-insoluble, lipid-free material was plated and assayed for radioactivity as previously described by Devi, Lerman and Sarkar[1] in the gas-flow windowless counter with an efficiency of 30 per cent.

In experiments where the incorporation of ^{14}C-uridine into RNA was studied *in vitro*, the lenses of 10 such rats were pooled and homogenized at 0° C. in 10 ml. of cold 0·25 *M* sucrose medium containing 0·025 *M* potassium chloride, 0·035 *M* potassium bicarbonate and 0·004 magnesium chloride[8]. The reaction mixture

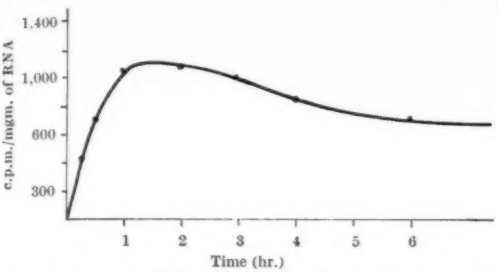

Fig. 1. Carbon-14 uridine incorporated into rat lens RNA (*in vivo*)

containing 0·5 ml. lens homogenate, 2 μmoles of adenosine triphosphate (ATP), 2 μmoles of Mg^{++}, 20 μmoles of *tris* buffer, *p*H 8·0, and 0·05 ml. 2-^{14}C-uridine, specific activity 0·55 × 10^6 c.p.m. in a total volume of 1 ml. was incubated for 30 min. at 37° C. The reaction was terminated by adding 4 ml. of cold 5 per cent TCA. The procedures for washing, plating, and counting were similar to those described above. In some experiments the insoluble material washed three times with TCA was heated in 5 per cent TCA at 90° for 15 min. and then centrifuged. 1 ml. of the TCA-extract obtained was dried and afterwards assayed for radioactivity. ^{14}C-uridine, when injected into rats, appreciable counts can be recorded in TCA-insoluble material. The incorporation reaches the peak within 60–90 min.; then very slowly and gradually declines. Even 24–48 hr. after injection, TCA-insoluble material shows 60–70 per cent of the radioactivity noted at 60–90 min. These results are shown in Fig. 1.

The ability of the lens homogenate to catalyse the incorporation of ^{14}C-uridine into lens RNA under various experimental conditions is shown in Table 1. This system requires ATP and is dependent on Mg^{++}. Omission of any one of the components from the reaction mixture results in a poor incorporation of ^{14}C-uridine into RNA. Incubation of the homogenate with (50 μgm./ml.) crystalline pancreatic ribonuclease (RNase) (Worthington) for 30 min. prior to the addition of ATP, Mg^{++}, ^{14}C-uridine and *tris* buffer renders the system inactive. If, on the other hand, the lens homogenate is first treated with 50 units of venom phosphodiesterase (Biochemical Research) and then incubated with the other components of the reaction mixture, the extent of uridine incorporation into RNA remains practically the same.

The lens homogenate, on exhaustive dialysis at 2–4° C. against 0·02 *M tris* buffer *p*H 7·4 containing 0·01 *M* ethylenediamine tetraacetate (EDTA), becomes inactive in catalysing the incoporation of ^{14}C-uridine into RNA, but the activity can be restored by adding Mg^{++} (Table 2). Mn^{++} can replace Mg^{++} to the extent of 70–80 per cent and Co^{++} to the extent of 35–40 per cent, while other divalent metallic ions such as Zn^{++}, Ca^{++} and Fe^{++} are comparatively poor activators. These metals do not, however, exert

Table 1. Incorporation of ^{14}C-Uridine into Rat Lens RNA (*in vitro*)

Components of the reaction mixture	c.p.m./mgm. of RNA
Complete system	11,650
Complete system − Mg^{++}	2,060
Complete system − ATP	4,625
Complete system + RNase (50 μgm./ml.)	2,850
Complete system + venom phosphodiesterase (50 units/ml.)	9,210

The complete system contained 0·5 ml. of dialysed rat lens homogenate, 2 μmoles of ATP, 2 μmoles of Mg^{++}, 20 μmoles of *tris*, *p*H 8·0 and 0·05 ml. of ^{14}C-uridine, specific activity 0·55 × 10^6 c.p.m.

Table 2. Effect of Metallic Ions on the Incorporation of ^{14}C-Uridine into Rat Lens RNA

Metals	c.p.m./mgm. of RNA	
(Mg^{++}, 2 μmoles, other metals, 5 μmoles)		
1. Lens homogenate + ATP + Mg^{++}	11,680	
2. Lens homogenate + ATP + Ca^{++}	2,250	
+ Mg^{++}		11,025
3. Lens homogenate + ATP + Fe^{++}	2,040	
+ Mg^{++}		10,965
4. Lens homogenate + ATP + Co^{++}	4,080	
+ Mg^{++}		12,005
5. Lens homogenate + ATP + Mn^{++}	9,294	
+ Mg^{++}		12,608
6. Lens homogenate + ATP + Zn^{++}	2,060	
+ Mg^{++}		10,985

any inhibitory effect as the extent of incorporation of carbon-14 into RNA remains the same in the presence of these metals provided Mg^{++} is also present. All these divalent metallic ions begin to exhibit an inhibitory effect when their concentration is raised above 10 μmoles in the test system. It should be of some interest to recall and compare the remarkable effect of Co^{++} in promoting the incorporation of amino-acids into lens RNA and proteins and the strong inhibition of the Co^{++} effect exerted by Ni^{++} (ref. 1) with the effect these metals have on this system which lends support to the conception that Co^{++} is highly specific in protein synthesis.

The addition of chloramphenicol (50–100 μgm./ml.) does not block the incorporation of ^{14}C-uridine into RNA; but the addition of 5-bromouracil, 5-fluorouracil or 2-thiouracil (at a level of 40–50 μgm./ml.) inhibits the incorporation only to the extent of 25–35 per cent. No further inhibition is noted with higher concentration of these analogues of this pyrimidine. The purine analogues such as 8-azoguanine and 8-azoadenine cannot inhibit the incorporation of uridine into RNA more than 20–30 per cent.

These results clearly demonstrate the existence of an active system in rat lens capable of synthesizing RNA. Previously it was possible to demonstrate the presence of an active protein-synthesizing system in rat lens[1]. These results seem to be consistent with the assumption that in rat lens and also in lenses of other species of animals metabolism of proteins and nucleic acids is an active dynamic process.

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Serum Lipids in Relation to the Intake of Alcohol and Sodium Chloride

The question whether variation in the intake of alcohol or sodium chloride has any marked influence on serum lipids is of considerable interest in human pathology. Whereas several investigators have studied the long-term influence of these factors, especially on vascular changes, relatively few reports have been

Table 1. Serum Lipids in Relation to Ingestion of Ethyl Alcohol

Patient, age and sex	Diagnosis	Equiv. dose of 100 per cent alcohol (gm.)	Time	Total lipid (mgm./100 ml.)	Cholesterol (mgm./100 ml.)	Phospholipids (mgm./100 ml.)	Blood alcohol (parts/1,000)
A.J. 86 *F*	Cerebral arterio-sclerosis	45	8.00	840	299	340	
			9.00	855	297	331	1·53
			12.00	815	289	330	0·68
			14.00	855	292	321	0·29
P.T. 84 *M*	Bronchitis	45	8.00	695	241	269	
			9.00	705	246	279	0·91
			12.00	715	244	253	0·32
			14.00	710	248	262	0·00
B.L. 30 *M*	Normal	45	8.00	525	188	192	
			9.30	525	186	178	0·45
			12.30	620	223	229	0·00
J.A. 82 *M*	Coronary sclerosis	67·5	8.00	780	284	283	
			9.00	690	255	255	1·30
			12.00	650	239	272	0·57
			14.00	770	263	289	0·24
J.J. 71 *M*	Hypertrophy of the prostate	45	8.00	625	205	240	
			9.15	670	205	253	1·09
			12.00	690	207	259	0·70
			14.00	685	221	263	0·42
C.J. 72 *M*	Coronary sclerosis	45	8.00	495	181	208	
			9.00	555	199	215	0·78
			12.00	465	170	201	0·26
			14.00	455	187	223	0·00
C.J. 72 *M*	Coronary sclerosis	45	7.50	470	168	168	
			9.30	340	141	149	0·67
			12.00	420	158	168	0·17

Table 2. Serum Lipids in Relation to Low- and High-intake of Sodium Chloride

Patient, age and sex	Diagnosis	Sodium chloride (gm./day)	Date of beginning and and end of regimen	Date of blood sample	Total lipid (mgm./100 ml.)	Serum: Total cholesterol (mgm./100 ml.)	Phospho-lipids (mgm./600 ml.)
N.C. 69 *M*	Senile dementia	Normal	–Nov. 15	Nov. 13	650	270	235
		0·5	Nov. 16–Nov. 29	Nov. 20	670	259	248
				Nov. 27	645	265	256
		8	Nov. 30–Dec. 13	Dec. 4	555	238	213
				Dec. 11	625	252	221
		Normal	Dec. 12–	Dec. 18	590	244	215
K.S. 74 *M*	Sequelæ hæmorrh. cerebri	Normal	–Nov. 15	Nov. 10	515	199	214
		0·5	Nov. 16–Nov. 29	Nov. 17	505	180	206
				Nov. 24	535	173	196
		8	Nov. 30–Dec. 13	Dec. 1	580	188	214
				Dec. 8	470	187	210
		Normal	Dec. 14–	Dec. 15	495	165	173
V.K. 79 *M*	Hypertension	Normal	–Nov. 22	Nov. 20	520	167	176
		0·5	Nov. 23–Dec. 6	Nov. 27	550	197	207
				Dec. 4	570	205	223
		8	Dec. 7–Dec. 20	Dec. 11	630	213	215
				Dec. 18	580	222	229
		Normal	Dec. 21–	Jan. 5	520	186	214
T.N. 89 *F*	Arteriosclerosis	Normal	–Nov. 22	Nov. 17	575	202	249
		0·5	Nov. 23–Dec. 6	Nov. 24	620	229	225
				Dec. 1	575	189	192
		8	Dec. 7–Dec. 20	Dec. 8	515	196	218
				Dec. 15	570	202	222
		Normal	Dec. 21–	Jan. 5	520	186	216

published on the variations in serum lipids caused by a single dose of alcohol and by short periods of high or low intake of sodium chloride. The present communication deals with some experiments aimed at elucidating these problems.

Experiments with alcohol. Six persons participated in this experiment : 1 normal person, 30 years of age, and 5 geriatric patients. One dose (100–150 ml.) of brandy (45 per cent v/v) was given at 8 a.m. to the fasting person together with 4 plain biscuits (24 gm.). Blood samples were taken immediately before and 1, 4, and 6 hr. after. Total lipids, cholesterol and phospholipids were determined in all the samples. Alcohol was determined in the samples after dosing, determinations being carried out at the Institute of Forensic Medicine, University of Copenhagen, by courtesy of Prof. H. Gormsen.

Furthermore, in all samples prothrombin-proconvertin, serum transaminase, alkaline phosphatases and icterus index were determined, and thymol- and Takata-Ara tests were carried out. The liver function as judged by these examinations was normal and did not show any variation during the experiment.

The results of the serum lipid and alcohol determinations are presented in Table 1. In no case were significant changes in total lipids, total cholesterol and phospholipids observed after the ingestion of ethanol in the quantities used. This seems to be in agreement with observations of Grande *et al.*[1], who did not find any significant increase in serum chole-

sterol when 0·45 gm. ethanol/kgm./day was given for three weeks, and an increase of only 18 mgm. cholesterol per 100 ml. serum when 1·35 gm./kgm./day was given for one week. Under quite different conditions, namely acute alcoholism with damage to the liver, Epstein and Greenspan[2] found a reduction of the cholesterol esters in blood plasma.

Experiments with sodium chloride. Four geriatric patients participated in this experiment. They were given a diet low in sodium chloride (less than 0·5 gm. sodium chloride/day) for two weeks and thereafter the usual hospital food (5 gm. sodium chloride/day) plus 3 gm. sodium chloride in tablets/day for the following two weeks.

Serum total lipids, total cholesterol and phospholipids were determined once before the experiment, twice during each experimental period, and once after the experiment. The results were presented in Table 2. The values for total lipid, total cholesterol and phospholipids did not change significantly from one period to the other. This finding does not necessarily contradict the possibility that a diet rich in sodium chloride may contribute to the development of atheromatosis, as has been claimed, for example, in rats[3].

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Eucollagen

As protein chemistry becomes more complex and large units become molecularly subdivided, new terms are required for adequate description. Prefixes such as α-, β-, pseudo- are well known. In collagen research, terms have been invented to cover new concepts, such as Orekhovich's procollagen[1], Banga and Balo's metacollagen[2] and the tropocollagen units of Schmidt, Gross and Highberger[3].

None of these terms was adaptable to the highly modified forms of collagen described by me[4], and so the term 'eucollagen' was used[5] in this connexion.

The *Shorter Oxford English Dictionary* describes 'Eu' as a prefix representative of Greek, used mostly in words of Greek derivation. εὐ κόλλα λεν is derived wholly from the Greek and clearly means 'easily producing glue'. This is an exact property entering into the definition and the term was chosen for this precise reason.

Unaware, at the time, of the original publication[5] on eucollagen and of subsequent references[6] to this term by Kelly, Little and Courts; Reed and Stainsby Crosby and Stainsby; and by Tristram, a second interpretation of eucollagen was used by [the late] W. T. Astbury[7] to name a hypothetical limiting structure for collagen, in which glycine occurs at intervals of three residues and hydroxyproline at intervals of ten residues. This unit is considered to be responsible for the 28·6 Å. X-ray repeat.

So far as the modified collagen[5] is concerned, many of the properties suggest a retention of the collagen helical configuration but absence of segmented spacings. A substantial reduction in molecular chain weight to parent gelatin size occurs. The hydrothermal stability falls to a minimum value and the material undergoes transition to a random coil structure entirely by a hydrogen bond-breaking mechanism. It is suggested, therefore, that use of the term 'eucollagen' for such material is both etymologically and scientifically in order.

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Some Properties of the Deoxyribonucleic Acid of Phage Alpha

WE wish to report some results concerning the unique properties of the deoxyribonucleic acid (DNA) isolated from the phage α grown on its host, *Bacillus megatherium*, strain Paris. This phage and its host have been studied by Drs. S. Aurisicchio, A. Celano, A. Coppo, P. Donini and F. Graziosi, and kindly supplied to us by them. These workers had found that the decay of phosphorus-32 incorporated in the DNA of the phage led to a time-dependent loss of viability similar to that of the phage φ*X*-174 (Graziosi,, F., personal communication). Since the DNA of the latter phage has been shown to be single-stranded[1], the DNA of phage α was expected to display similar properties. However, as will be indicated later, many of its properties resemble those of double-stranded DNA with the notable exception that, on denaturation, there are formed two denatured units with distinct buoyant densities in a cæsium chloride gradient.

After gathering and purification of phage α, DNA was isolated by the phenol method of Mandell and Hershey[2]. Using as much caution as possible to avoid shear degradation, a mean sedimentation coefficient of 40*S* (extrapolated to infinite dilution and corrected to water at 20° C.) was obtained for this type of preparation. Exposure to heat resulted in an absorbance–temperature profile similar in the sharpness of its rise of absorbance to that of the DNA of the *T* phages of *Escherichia coli*, with a T_m (midpoint of the rise of absorbance) of 86·5° C. in 0·15 *M* sodium chloride p'us 0·015 *M* sodium citrate. This T_m corresponds to a base composition of 41 mole per cent guanine plus cytosine (ref. 3 and Marmur, J., and Doty, P., unpublished work). When phage α DNA is denatured and then renatured under optimal conditions[4], it exhibits an absorbance–temperature profile similar to that of the native; the curves can be superimposed to the extent of approximately 85 per cent in the region of the T_m.

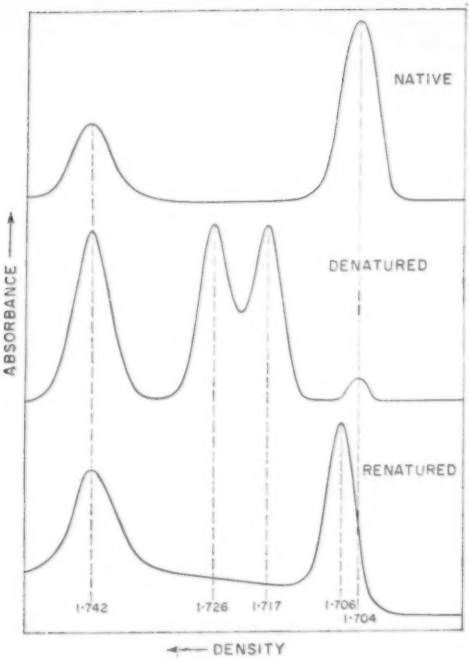

Fig. 1. Buoyant densities of native, denatured and renatured phage α DNA. Microdensitometer tracings were made of the ultra-violet absorption photographs taken after 23 hr. at 44,770 r.p.m. (25° C.). The density 1·742 represents that of the standard, *Pseudomonas aeruginosa* nitrogen-15 DNA, which is used to calculate the densities of the other bands

Native and denatured samples of phage α DNA were then examined for their properties in cæsium chloride density gradient centrifugations. The results are shown in Fig. 1. Native phage α DNA was equilibrated in a single band with a buoyant density of 1·704 gm./c.c. This density corresponds to a base composition of 44 mole per cent guanine plus cytosine (refs. 5, 6 and Schildkraut, C. L., Marmur, J., and Doty, P., unpublished work). However, a thermally denatured aliquot gave rise to two equal distinct bands with densities of 1·717 gm./c.c. and 1·724 gm./c.c. When the denatured material was renatured, again one band was obtained. The density of this band (1·706 gm./c.c.) is slightly higher than that of the native DNA, a result most likely due to the persistence of some denatured unmatched ends.

The two distinct denatured units, obtained on thermal denaturation (and on formamide denaturation as well), might result from a variety of factors. The two hypotheses which at the present time we favour as explanations of these results may be summarized as follows: (1) That there is a difference in the base compositions of the two units or strands which causes one or both of them to have a different density from that usually obtained for a DNA of this overall base composition (42·5 per cent, obtained by chemical analysis, Graziosi, F., personal communication). The Watson–Crick structure does not exclude this possibility even for deoxyribonucleic acids in which adenine = thymine and guanine = cytosine (as has been shown for this DNA, Graziosi, F., personal communication). (2) That an unknown material, *X*, is attached to one of the two units causing it to have a lighter or heavier buoyant density.

In considering the first hypothesis, it is possible to calculate the percentage of guanine plus cytosine for each of the denatured bands from their buoyant densities. This corresponds to 42 mole per cent for the lighter and 49 mole per cent for the heavier band. The lighter denatured band then has, by this calculation, a guanine plus cytosine content closely approximating that obtained by chemical analysis and by the T_m of the absorbance–temperature profile. However, the average composition calculated from the densities of the two bands (45 per cent) is close to that corresponding to the buoyant density measured for the native material. Since the lighter denatured band approximates the average base composition of the double-stranded material, we feel inclined to favour hypothesis (2). Some unknown material firmly attached to one of the strands causes it to exhibit a higher buoyant density, resulting in the two-band pattern observed by thermal and formamide denaturation. If only one of the two strands of the DNA molecule were essential for the replication of the phage, it would then be possible to explain the high phosphorus-32 suicide of the phage.

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An Inhibitor of Liver Alcohol Dehydrogenase in Preparations of Reduced Diphosphopyridine Nucleotide

Different samples of reduced diphosphopyridine nucleotide (β-DPNH, Sigma Chemical Co.) have been found to give significantly different maximum rates of reduction of aldehyde catalysed by liver alcohol dehydrogenase. The crystalline enzyme was prepared from horse liver[1] and measurements of initial rates were made with a recording fluorimeter[1,2] at 23·5°. In Table 1 relative values of initial rates with $1·3 \times 10^{-3}$ *M* acetaldehyde and 2×10^{-4} *M* DPNH in *p*H 6 phosphate buffer (ionic strength 0·1) are collated with assays of the samples of DPNH. The percentage by weight of anhydrous disodium salt was calculated from direct extinction measurements at 340 mμ (column 2), and also from the decrease of absorption of light at this wave-length ($\Delta E340$) accompanying complete oxidation of the coenzyme by excess acetaldehyde and liver alcohol dehydrogenase (column 3), using in each case the value 6·22 (ref. 3) for the millimolar extinction coefficient. The second value is taken to be the true DPNH content. The difference between the two values is due to the presence of a substance which closely

resembles DPNH in both light absorption and fluorescence emission, but is not coenzymically active with liver (or yeast) alcohol dehydrogenase. The results indicate that the samples which give the higher initial rates have the greater coenzyme content and a smaller excess of total adenine over coenzyme (column 4). Ribose and total phosphate analyses were also made ; calculating the total adenine content from the extinction at 260 mμ (*p*H 7) and a millimolar extinction of 15 (ref. 4), the molar ratios of ribose and phosphate to adenine were 2·1 ± 0·1 for all the samples.

With butyraldehyde as substrate, similar differences in initial rates at *p*H 6 were found. At *p*H 7 the differences were smaller, and with acetaldehyde at *p*H 10 all the DPNH samples gave the same initial rates. With yeast alcohol dehydrogenase (Worthington Biochemical Corporation) the rates of reduction of acetaldehyde at *p*H 6 were the same for all the DPNH samples. It appears that the more impure samples at least contain a potent and relatively specific inhibitor of liver alcohol dehydrogenase in neutral and acid solution.

Table 1. Assay Values and Relative Initial Rates of Oxidation of DPNH Samples by Acetaldehyde and Liver Alcohol Dehydrogenase at *p*H 6·0

Sample	Assay, percentage DPNH(Na_2)		$E260/\Delta E340$	Initial rate (arbitrary units)
	$E340$	$\Delta E340$		
1*	73	66	3·6	57
2	78	73	3·0	68
3	85	80	2·85	96
4	88	81	2·75	100

* Sample 1 not designated as the β-isomer.

The following compounds were tested as inhibitors with negative results : nicotinamide and its oxidized and reduced N-methyl derivative, adenosine, adenosine di- and tri-phosphate, yeast adenylic acid, and α- and β-DPN. Digests of DPN and DPNH with nucleotide pyrophosphatase[5], which yield adenosine monophosphate and nicotinamide mononucleotide, were also not significantly inhibitory. Treatment of DPNH with 0·1 *N* hydrochloric acid and DPN with 0·1 *N* sodium hydroxide produced potent inhibitors, however ; both these treatments form adenosine diphosphate ribose[6], and this compound was isolated from acidified DPNH and from digests of DPN with the nucleotidase of *Neurospora crassa*[7]. A concentration of 8×10^{-6} adenosine diphosphate ribose produced 40 per cent inhibition in the rate of reduction of acetaldehyde at *p*H 6 under the conditions described here and using the purest DPNH preparation, but had no inhibitory effect at *p*H 10. It did not affect the reaction catalysed by yeast alcohol dehydrogenase at *p*H 6. Digestion of the compound with nucleotide pyrophosphatase destroyed its inhibitory power. Direct evidence of the presence of adenosine diphosphate ribose in DPNH samples has been obtained by paper electrophoresis, but it is not yet possible to say whether the amount is sufficient to account for the observations.

The remarkably potent and *p*H-dependent inhibition of liver alcohol dehydrogenase by adenosine diphosphate ribose has obvious possibilities for work on coenzyme binding. It also seems probable that discrepancies between the kinetic data for this enzyme[1,8,9], which were particularly large as regards the maximum rate of aldehyde oxidation in acid solution, were mainly due to the presence of different amounts of inhibitor in the coenzyme samples. Highly purified coenzyme samples are evidently essential for valid kinetic studies, especially if conclusions about the mechanism are to be drawn from relations between the forward and reverse rates and equilibrium constants[8,10,11]. It remains to be seen whether these observations and conclusions are relevant to other dehydrogenases.

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A New Universal Buffer System

During investigations of the effect of *p*H on the activity of certain enzymes it was found necessary to use a single buffer solution for measurements at widely different *p*H values. For various reasons the universal buffer solutions of Prideaux and Ward[1], and Britton and Robinson[2] were unsuitable, and so the following solution was devised, the *p*H value of

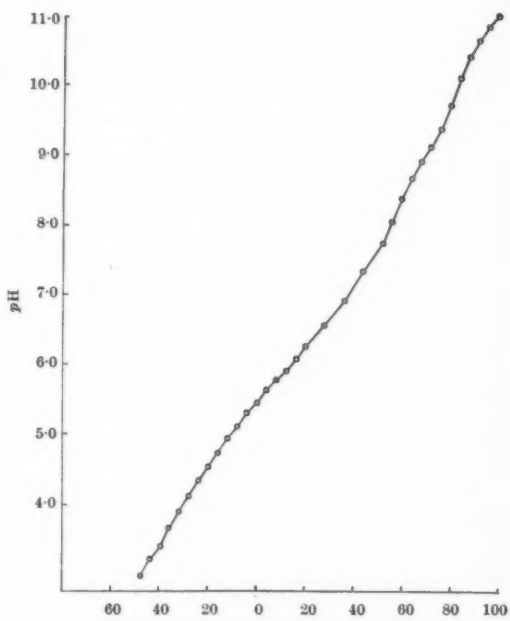

Fig. 1

which was found to vary almost linearly with added acid or alkali.

0·1 mol. of each of the following was dissolved separately in 250 ml. water, the solutions being mixed in the order shown just prior to use: sodium carbonate, 2-amino-2-methyl propan-1 : 3-diol[3] (Eastman-Kodak reagent); sodium dihydrogen orthophosphate; citric acid.

Van Slyke[4] and Britton and Robinson[2] deduce that in mixtures of buffer substances a constant buffering power over the working *p*H range will be obtained if the consecutive *pK* values of the acids involved differ by not more than 1·2. In the present system the *pK* values are deduced to be[2,5]:

1·0; 3·1: 4·7: 5·4: 6·4: 7·1; 8·8; 10·4 and 12·3.

These approximate to the ideal system, and the buffering power in fact remains nearly constant between *p*H 2 and 12 (Fig. 1).

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RADIOBIOLOGY

Determination of Cæsium-137/Potassium Ratios in Diet and in the Human Body by Urine Analysis

A radiochemical method[1] has been developed for the determination of cæsium-137 in urine. Acidified urine containing cæsium carrier is passed through a column of ammonium phosphomolybdate mixed with an equal weight of asbestos to give adequate porosity. The ammonium phosphomolybdate acts as a highly selective cation-exchanger and retains the cæsium quantitatively, even at comparatively high flow-rates. Other cations, including sodium, potassium and the alkaline earths, are not retained to any significant extent. After all the sample has passed through the column, the ammonium phosphomolybdate is dissolved in sodium hydroxide solution and the cæsium precipitated as the cobaltinitrite. The removal of final traces of potassium and rubidium from the cæsium is ensured by its subsequent precipitation as the bismuth iodide complex and finally as the chloroplatinate, which is filtered, dried and beta-rays counted. The recovery of carrier normally exceeds 80 per cent. The sensitivity of this method makes it particularly useful for the determination of fall-out cæsium-137 in urine, as current concentrations are low and are likely to decline still further.

The cæsium-137 contents of seven urine samples collected in February, March and April 1961 were determined by this method. These samples were prepared by bulking at least 10 specimens obtained from individuals, mostly resident in Berkshire, with no known exposure to cæsium-137 other than fall-out levels in their diet. Potassium was estimated in the same samples by flame photometry. The results of these analyses are given in Table 1.

Table 1. Cæsium-137 and Potassium Concentrations in Bulked Urine Samples

Date of collection	Cæsium-137 (μμc./l.)	Potassium (gm./l.)	Ratio (μμc./gm.)
21.2.61	29·9	2·47	12·1
1.3.61	21·1	2·00	10·6
7.3.61	26·8	2·73	9·8
14.3.61	24·3	1·73	14·0
20.3.61	24·6	1·66	14·8
10.4.61	26·2	2·07	12·7
28.4.61	21·7	2·07	10·5
	Mean 24·9	Mean 2·10	Mean 12·1

In vivo cæsium-137/potassium ratios were determined by Rundo, J. (private communication, 1961) in 14 residents of Oxfordshire and Berkshire during March 1961. The mean value for this group was 36 μμc./gm., which is almost exactly three times the value for this ratio in urine given in Table 1. Values for the ratio :

$$\frac{\text{cæsium-137/potassium in the body}}{\text{cæsium-137/potassium in urine}}$$

have also been determined for a number of individuals by Rundo and by Miller and Marinelli[2]. These varied from 1·9 to 4·6, indicating that individuals may show a considerable departure from the average value of 3 obtained in the present comparison.

As both cæsium and potassium are completely absorbed from the human gastro-intestinal tract, the cæsium-137/potassium ratio in urine will be equal to that in the diet and should also be one-third of that in the body, under equilibrium or near equilibrium conditions. This is in agreement with the measurements of McNeill and Trojan[3], who measured the cæsium-137/potassium ratio in the diet of Toronto residents and in the residents themselves, using a γ-spectrometric technique. They found that the body/diet ratio was 3: 1.

The analysis of bulked urine samples appears to provide a simple method for assessing current cæsium-137/potassium ratios in both diet and the human body, so long as dietary-levels are not changing too rapidly. By analysing a bulked sample, the difficulty in selecting representative aliquots of various items of diet for analysis is avoided.

The possibility of monitoring strontium-90 in diet by urine analysis has been proposed by Schulert[4], whose measurements show that the strontium-90/calcium ratio in diet is approximately twice that in the urine. Both strontium-90 and cæsium-137 may be estimated on the same sample by adding strontium and cæsium carrier to urine before passing it through the ammonium phosphomolybdate column. While cæsium is retained on the column, both strontium and calcium pass through and may be co-precipitated from the effluent as the oxalates. Separation of the strontium from the calcium and determination of the strontium-90 can then be effected by well-established techniques.

We wish to thank Mr. W. H. A. Raymond of the Medical Research Council Radiobiological Unit, Harwell, who carried out potassium determinations in the urine samples.

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Effect of Irradiated Normal Tissues on the Growth of Mouse Carcinoma

In 1932 it was observed by Geoffrey Keynes[1], working on the disappearance of human mammary carcinoma by surrounding the growth with radium needles, that on cutting out a piece of the tumour after the needles had been in place for ten days, some of the malignant cells were alive and became grafted in the overlying biopsy skin-incision, although the rest of the tumour from which the piece had been removed eventually disappeared. This suggests that the surrounding normal cells might have been acted on by the radiations so as to continue to pour out a chemical substance which led to the death of the cancer cells[2].

It was decided to search for evidence of the presence of such substances in irradiated normal tissues. The material tested has been bovine and rabbit muscle, lamb's thymus and mouse meat. The irradiated tissue was supplied either solid as food or in water as a drink. The tumour used was one of two mice carcinomata, Bashford 63 (also labelled *X*), or Compton 22, which arose in our laboratory spontaneously and so far has not been described. The general method of experimenting was to implant grafts from the same fragment of tumour subcutaneously into 10 mice. From these animals, 1–5 were taken at random for testing the irradiated material. The others were kept as controls. Both control and test animals were fed on the pellets customarily used in laboratories, but the test animals had either (*a*) the irradiated material, if solid, added to the ground-up pellets or (*b*) had a watery extract of the irradiated material substituted for their drinking water. There was no restriction of the amount the animals were allowed to eat or drink.

Experiments showed that unirradiated meat or meat extracts added to the normal diet of the mice had no effect on tumour growth; and that animals receiving irradiated food showed no alteration in weight compared with the controls.

In 23 experiments of this nature, 16 by the feeding procedure and 7 by administering the material by drinking, the results were as follows: (*a*) in 6 there was no evidence of inhibition; (*b*) 17 showed disappearance of the growth or inhibition in some degree.

Among the failures was a drinking experiment where the material had been heated to 80° C. In three others the material had received doses of 1,000 r., 6,000 r. and 7,500 r., which we consider as inadequate, while in one, although the dose given to the food 120,000 r. was suitable, the animal was fed for only 3 days. For the sixth failure we have no explanation.

Experiments 619 (feeding with lamb's thymus, radiation dose 600,000 r.) and 664 (drinking experiment with a watery extract of beef muscle, radiation dose 150,000 r.) are examples of findings.

Experiment 619 (Fig. 1) illustrates the disappearance of 3 treated tumours and, while feeding was proceeding, inhibition in 2 others.

In the experiment 664 (Fig. 2), No. 2 was treated from the fifth day after implantation of the graft, for 7 days. The tumour disappeared. No. 1 was allowed to grow until the eleventh day and then given the drink for 7 days. It showed some initial shrinking in size but then resumed growth and caught up with the controls by the eighteenth day. This suggests that there is a simple relation between the amount of active agent necessary to cause disappearance of the growth and the weight of the tumour (proportional to r^3 in the case of a sphere). Below this critical amount, inhibition only can occur and the growth of the tumour soon gets the upper hand. The amount of active agent in our extracts is likely to be small, so that the animal may not drink enough to absorb the critical amount for disappearance of the tumour.

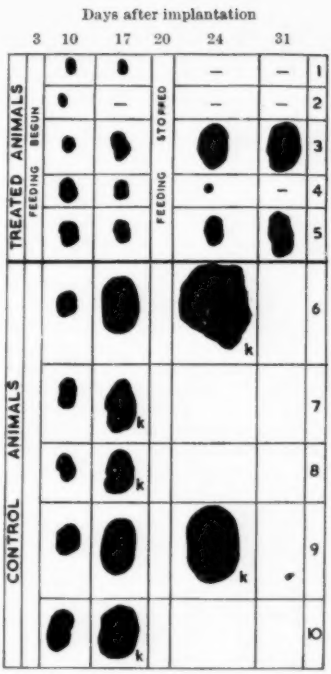

Fig. 1. Experiment 619, tumour 63. *K*, killed for tumour propagation purposes

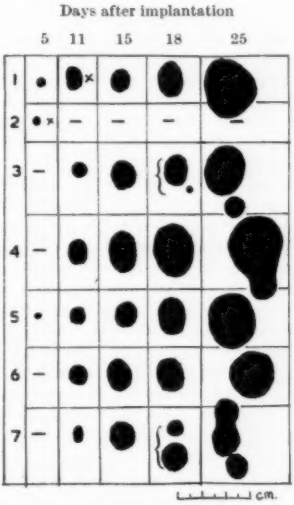

Fig. 2. Experiment 664, tumour *X*. *X*, treated animals ; the other animals are controls

Graphs of the rate of tumour growth in controls and in treated animals where the tumour is inhibited but does not disappear show the same general character; but the growth-time curve for the inhibited tumour shows a variable delay before it turns upward.

Assuming the tumours to be spherical in shape and that the inhibitory effect is due to direct destruction of tumour cells, theoretical curves have been plotted for different amounts of active agent given. It appears that for complete disappearance of a tumour a dose is required which will destroy at least 37 per cent of it.

The inhibitor seems to be diminished in activity by heating above 60–65° C. and destroyed by an X-ray dose greater than from 10^6 to 2×10^6 r.

A similar phenomenon in plants has been reported from the U.S.S.R. A. M. Kuzin[3] has found evidence that irradiating the normal cells of a plant produced substances which had an inhibitory effect on cell division in other parts of the plant which had not been exposed to X-rays. He isolated from the irradiated part a mixture of polyphenols which proved to be the active substance.

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PHYSIOLOGY

Spontaneous Spike-Discharges of Vascular Smooth Muscle

I reported in refs. 1 and 2 that trans-membrane potentials of smooth muscle fibre of the small blood-vessels, distributed in frog tongue and web, had been studied with the aid of an intracellular micro-electrode technique. The results of these experiments were as follows: (1) The resting potential of smooth muscle fibres was found to be negative on the inside, against the external medium; the mean value was $-40{\cdot}5 \pm 5$ mV. and fluctuations of the resting potential were rarely observed. (2) Single spike-potentials were evoked by single induction shocks, which were about 60 mV. in magnitude and 200 msec. in duration.

Though these potentials usually had the shape of simple spikes, some of them showed the so-called 'complex shape'[3,4] provided with some humps on the phase of either depolarization or repolarization, or on both, and showed graded response. Another type of action potential was obtained from smooth muscle fibres in the remarkably contractile region of the bifurcation of small blood-vessels or in blood capillaries that were highly sensitive to electrical or chemical stimulation; in this type the magnitude was about 60 mV. and the duration 50 msec. This evidence suggested that there were two sorts of muscle fibres, distinguishable in contractility, in the small blood-vessel system.

(3) The mean value of the resting potential of endothelial cells in blood capillary wall was $-47 \pm 3{\cdot}7$ mV., but spike-potential, elicited by electrical stimulation, had not been observed.

In the present work, the spontaneous spike-discharges of vascular smooth muscle of frog were observed with the intracellular micro-electrode. The recordings *in situ* were carried out in the following

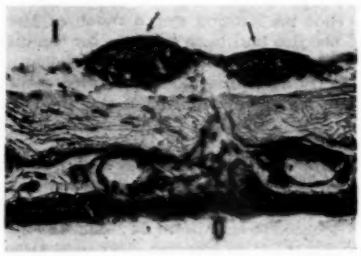

Fig. 1. Photomicrograph showing a transverse section of a blood-vessel in the skin of the lateral abdomen of a frog. Note the absence of visible connective tissues in the surface of the blood-vessels. The arrows mark blood-vessels (venules containing red-stained corpuscles). *I* and *O* are the inside and outside respectively of the skin. Stained with hæmatoxylin–eosin. (× 100)

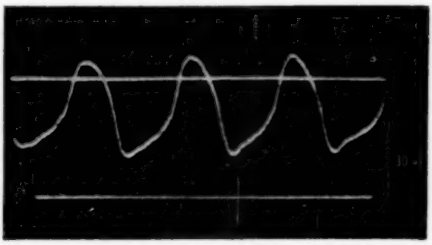

Fig. 2. A series of the spike-discharges recorded intracellularly from a vascular smooth muscle fibre (venule). In this record the resting and action potential are −26 and 36 mV. respectively. The spikes show an overshoot of about 10 mV. and a time-course of about 200 msec. Note the initial slow potential (pre-potential), from the summit of which the spike arises. The horizontal line indicates zero potential. Calibration, 30 mV.; time mark, 30 msec.; temperature, 29° C.

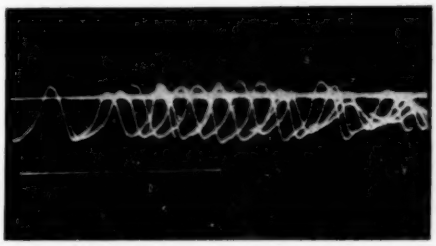

Fig. 3. Typical record of spike-discharges obtained from a muscle fibre with an intracellular micro-electrode during spontaneous activity (arteriole). This continuous record was taken repeatedly with several sweeps on a slow-moving film. The horizontal line indicates zero potential. Calibration, 30 mV.; time mark, 30 msec.; temperature, 26° C.

Fig. 4. A vascular pulsation curve recorded from the same specimen as in Fig. 3, in which the period is about 900 msec. This curve is shown to compare with the time-course of the spike. Time mark, 30 msec.

way: the frog was immobilized by fixing the dorsal side down with a metal cover in a lighted chamber. The skin of the abdomen was incised both along the mid-line and at the flank so as to be turned over, and was spread out under moderate tension on a cork board immersed in Ringer solution. A micro-electrode was then inserted into the blood-vessel wall from the outside with the aid of a Peterfi-type micro-manipulator under the binocular microscope. The micro-electrode filled with 3 *M* potassium chloride solution was used to record the potential, a relatively high-resistance micro-electrode (35–50 MΩ) being used for the purpose of minimizing injury to the muscle cell. For the arteriole, the tip of a micro-electrode had to be thinner than for the venule, in order to penetrate the wall safely. For the convenience of good penetration small blood-vessels, with very thin connective tissue membrane covering the surface as shown in Fig. 1, of 50–100μ in diameter, were selected. The control of vascular pulsation was made possible by applying adequate pressure to the proximal part of the same blood-vessel at some distance from the point of impalement.

All experiments were carried out at temperatures between 25° and 32° C. When the tip of a micro-electrode penetrated into a muscle fibre of a small blood-vessel, the resting membrane potential showed a negativity against the external medium, the value of which was about − 25 mV. During the observation, repetitive bursts of spike-discharges occurred sometimes spontaneously at regular spike-intervals, and lasted for several seconds or longer. The typical record is shown in Fig. 2. In this record the resting and action potentials were − 26 and 36 mV. respectively, the resting potential being measured at maximum polarization between one spike and another. The action potentials had an overshoot of about 10 mV. and a time-course of about 200 msec. They were invariably provided with an inflexion of the rising phase. This indicated that the spike configuration consists of two components, namely, an initial slow potential and a large simple spike.

The slow potential resembled in appearance pre-potentials of other smooth muscles (tænia coli[3], pregnant uterus[5], etc.) or excitatory post-synaptic potentials of various neurones[6,7]. The spike-potential recorded appeared to be conducted excitation in view of the initial slow potential and it is probable that the excitation is propagated from one unit to another to some extent in the blood-vessel. It is with this point taken into account that the mechanism of vascular activity is now under investigation by me.

With the spike-discharges, there was a progressive decrease of the amplitude towards the end, finally reaching a certain depolarization-level of the resting potential. Though the spontaneous discharges were observed in both arterioles and venules, no significant difference was noted between them.

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Stimulation of Motor Nerve Terminals

Knowledge of prejunctional events in neuromuscular transmission is at present indirect in origin and largely speculative in content. This is because there has been no method of directly recording from, or stimulating, the very small motor nerve endings. Using a glass micro-electrode in the isolated rat diaphragm phrenic nerve preparation we have succeeded in stimulating single nerve fibres very close to, or at, the neuromuscular junction. The recording chamber and bathing solution have been previously described[1].

The stimulating electrode (filled with 4 *M* sodium chloride, 1·5–3μ tip and 1–3 megohm resistance) was located at neuromuscular junctions by using it to record extracellular end-plate potentials in curarized ($2–4 \times 10^{-6}$ gm./ml.) or magnesium-poisoned (11 m.mole/l.) preparations (Fig. 1*A*). With this method extracellular end-plate potentials are recorded only when the tip is very close to the junction. When localized in this way the electrode was connected to two Grass stimulators which delivered pairs of pulses separated by varying intervals (1–200 msec.). These pulses were recorded across a known small resistance in the stimulating circuit and monitored on the C.R.O. screen. In response to stimulation small all-or-nothing potentials could be recorded in the phrenic nerve (Fig. 1*B*). The position of the electrode tip was very critical in that movements of only 10–15μ sideways or upwards caused the response to stimulation to be lost. Moving the electrode downwards resulted in impalement of the muscle fibre. As the diameter of the neuromuscular junction in the rat diaphragm is 20–30μ (ref. 2) we feel that we are stimulating structures very close to the nerve terminals.

The changes in threshold after one supra-threshold conditioning stimulus have been examined in both magnesium-poisoned and curarized preparations. The results (Fig. 1*C*) indicate a refractory period of 1·5–3 msec. followed by a period of supernormality lasting 15–30 msec. There is often a slight subnormality for about 50 msec. after the supernormal period (Fig. 1*C*). Following 2–5 conditioning volleys the period of supernormality is reduced and the subnormality increased in magnitude and duration. These excitability changes of the terminals are very similar in time course to those described for the parent fibres, the mammalian *A* fibres[3]. After a brief (0·1 msec.) sub-threshold conditioning pulse changes in threshold—an initial fall and later recovery—can be detected for 2·5–3·0 msec. in both magnesium-poisoned and curarized preparations.

We have further found that the excitability of the terminals can be markedly altered by drugs. A comparison of magnesium and curare at the same neuromuscular junctions has shown that curare reversibly reduces the supernormal period found after one conditioning stimulus both in curarized and magnesium-poisoned preparations. Prostigmine in concentrations as low as 10^{-7} gm./ml. produces a reversible increase in refractory period followed by an increase in the magnitude and duration of the supernormal period (Fig. 1*C*).

These findings are of interest in two connexions. First, prolongation of the refractory period and thus presumably of the spike potential in the terminals suggests that part of the effect of prostigmine on the end-plate potentials is caused by a prolongation of

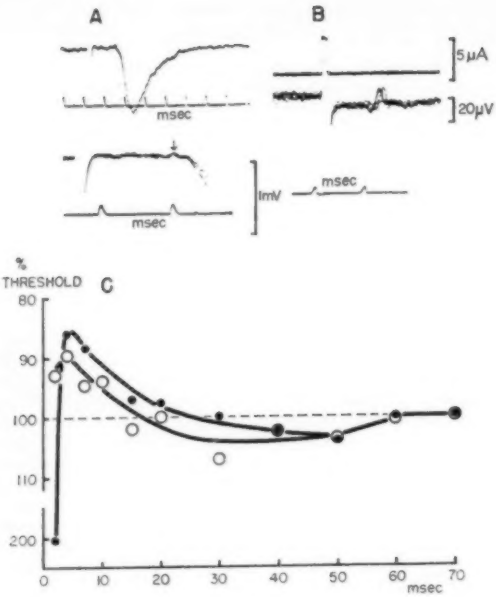

Fig. 1. *A*, Extracellular end-plate potentials recorded with a 4-*M* sodium chloride electrode, neuromuscular transmission being depressed by magnesium chloride 11 m.mole.; *B*, action potential in phrenic nerve in response to threshold stimulation (with square pulse of 0·06 msec. duration) at same neuromuscular junction as *1A*; *C*, changes in threshold after one conditioning stimulus. Ordinate: threshold current at given interval as percentage of unconditioned threshold current. Abscissa: interval between conditioning and testing pulses. ○, magnesium chloride 11 m.mole values before and after prostigmine bromide, averaged; ●, magnesium chloride 11 m.mole and prostigmine bromide 2×10^{-4} gm./ml.

the period of transmitter release. Secondly, the prostigmine-induced increase in negative after-potential provides an adequate mechanism for the initiation of the antidromic discharges found in motor nerves after intra-arterial administration of this drug[4].

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Potential Changes produced by Application of Current Steps in Motoneurones

In cat spinal motoneurones, when current steps are applied across the membrane through the intracellular micro-electrode, the membrane potential changes in an approximately exponential way, but with a certain deviation. Thus, the soma-dendritic membrane of the motoneurone has been assumed to behave as a resistance and a capacitance in parallel[1,2], and the deviation has been attributed to the cable-like property of dendrites, the dominance of which in forming the effective surface area of motoneurones has been emphasized recently[3]. As will be described, however, the potential produced by current steps in motoneurone membrane shows a considerable overshoot, which has so far been overlooked because the current steps used previously were generally too brief to reveal the later decline of the potential. This electrical behaviour of the membrane suggests that the equivalent electrical circuit of the membrane is not a simple resistance-capacitance network.

Single micro-electrodes filled with 3-mole/l. potassium chloride solution were inserted into motoneurones in the lumbosacral region of the cat spinal cord. Rectangular currents were applied to the impaled cell membrane through the micro-electrode, and changes in the membrane potential thereby induced were recorded through the same micro-electrode by compensating for the voltage set up across the micro-electrode resistance. The applied current was monitored by the potential drop across a 10-M.ohm resistor inserted in series between the micro-electrode and the 100-M.ohm resistor, the latter being connected to the current generator. After d.c. amplification, the potential changes in the membrane were recorded photographically from a cathode ray oscilloscope trace.

Fig. 1 *A* and *B* shows an example of the potential change thus induced by rectangular current pulses of about 50 msec. duration. The current strength was about half the rheobase for initiating the *IS–SD* spike potential. Both depolarizing (*A*) and hyperpolarizing (*B*) potentials rose in an approximately exponential way to a maximum at about 15 msec., there being a later decline during the continuation of the currents. After cessation of currents, the membrane potentials rebounded in the opposite direction before returning to the original level, as seen with slower sweep velocity in Fig. 1 *C* and *D*. In Fig. 1 *E* and *F* the potential induced by current steps reached a steady level in about 100 msec. after the summit at about 15 msec. The rebound, after current ceased, followed a course similar to that of the initial hump at the onset of the current. The potentials caused by depolarizing and hyperpolarizing currents were virtually mirror images. In these records, care was taken to eliminate distortion by electrode polarization[1]. Traces in Fig. 1 *G–L* were obtained just after the withdrawal of the micro-electrode to the extracellular position by applying the same currents used in *A–F*, and they showed that electrode polarization was not responsible for the overshoot at the current onset and the undershoot when current ceased. The net changes in the membrane potential are illustrated in Fig. 1 *N* by subtracting the extracellularly recorded electrode polarization from the corresponding intracellular potential in the same motoneurone as shown in Fig. 1 *E* and *F*. Measurements of the threshold changes give further evidence that these observed potential changes are taking place across the cell membrane. Short depolarizing pulses (duration, 0·2 msec.) were applied to the membrane through the micro-electrode. The threshold strength was measured as the current exciting the cell in about 50 per cent of the trials[1,2]. The decrease or increase of the threshold strength of pulses during the depolarizing or hyperpolarizing current steps is plotted in Fig. 1 *M*. As shown by dashed lines through the plotted points, the change of the threshold was closely related to the observed change in the membrane potential in *N*.

Changes in membrane potential resembling those of Fig. 1 have been observed constantly in all fifteen

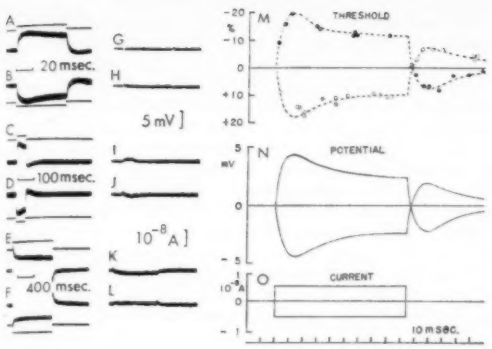

Fig. 1. *A* to *F*, depolarized (*A*, *C*, *E*) and hyperpolarized (*B*, *D*, *F*) potentials in the cat motoneurone membrane were induced by rectangular current pulses. The applied currents were registered in the companion traces of each record. *G* to *L*, electrode polarization recorded in the extracellular position by applying the same currents that were used for obtaining the intracellular records arranged to the left in *A*–*F*. *M*, Increase (+) and decrease (−) of the threshold strength of short pulses during conditioning by hyperpolarizing (○) and depolarizing (●) currents which are shown in O. *N*, change in the membrane potential by the conditioning currents. Note that records *A*–*D* were obtained in a different cell from that for *E*, *F*, *M* and *N*. The voltage and current calibrations apply to the appropriate records *A* to *L*

motoneurones examined so far. When current steps of from 2×10^{-9} amp. to 6×10^{-9} amp., in each case corresponding to about half the rheobase for each motoneurone, were applied, the polarized potential reached the summit at 16 msec. on the average (range, 10–22 msec.) after the onset and then declined by 30 per cent on the average (range, 17–46 per cent) of the peak value in the following interval of about 100 msec. This electrical behaviour of the motoneurone membrane may be explained by assuming that an inductive reactance is involved in the membrane, as has been proposed for squid giant axon[4,5], and for frog nerve[6]. Since the over- and under-shoots of polarized potentials are considerable, the analysis of the time-course of the rise or fall in potential caused by the current steps can no longer be based on the previously assumed resistance-capacitance model. The small undershoot following the monosynaptic excitatory post-synaptic potential, which summates to a large undershoot after a repetitive series of post-synaptic potentials[7], appears to be another example of the undershoot described here. During a depolarizing steady current, the change in membrane threshold is correlated accurately with the change in the membrane potential and is not strictly an example of accommodation of the membrane as has hitherto been assumed[1,2].

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Effects of Corticotrophin, Hydrocortisone and Methopyrapone on the Circulating Eosinophiles in Man

2-METHYL-1,2-*bis*(3-pyridyl)-1-propanone (methopyrapone) has been shown to stimulate the secretion of corticotrophin (ACTH) in the pituitary by inhibition of production of cortisol. 11-Desoxycortisol (compound *S*) is a precursor of cortisol and 11β-hydroxylation of compound *S* converts it into cortisol. Methopyrapone (*SU*-4885) inhibits 11β-hydroxylation of 11-deoxycortisol[1]. The result is the rise of compound *S* and fall of cortisol in the adrenal cortex and the blood. Secretion of corticotrophin or its administration by the parenteral route stimulates the adrenal cortex. The resultant release of hydrocortisone and other glucocorticoids from the adrenal cortex causes a marked eosinopenia. A clinical trial was undertaken to evaluate and compare the effects of exogenous administration of ACTH and hydrocortisone and endogenous secretion of corticotrophin by methopyrapone. This work is a part of our neuroendocrinological investigation of the role of methopyrapone and phrenotropic drugs in man[2].

Twenty normal human subjects volunteered for clinical trial. One group of five received 25 units of ACTH parenterally, the second group of five, 80 mgm. hydrocortisone equally divided, orally and parenterally, and the third group of five, 1 gm. methopyrapone orally and 1 gm. dissolved in 250 ml. 0·9 per cent saline infused in 1 hr. Five subjects acted as controls. Using phloxine as a staining medium, circulating eosinophile counts of the venous blood were done just before and 3 hr. after administration of the drug.

Table 1 shows the analysis of variance of control versus drugs and among drugs, and is significant in the comparisons. Tables 2–5 indicate the percentage fall in the circulating eosinophiles 3 hr. after the administration of the drug. The mean percentage fall was 83·3 with ACTH, 80·7 with hydrocortisone, 24·4 with methopyrapone and 1·8 in controls. In one control there was a rise of 22 per cent in the circulating eosinophiles.

Previous work has shown that the parenteral administration of 25 units of ACTH in man resulted

Table 1. Analysis of Variance of Changes in Eosinophile Count after 3 hr.

Source of variation	Degrees of freedom	Sum of squares	Mean squares
Treatments			
Control versus drugs	1	9,702·816	9,702·816
Among drugs	2	11,426·534	5,713·267
Individuals	16	17,943·6	1,121·475
Total	19	39,072·95	

$F1,16 = 8·651$ $P < 0·01$ (control versus drugs)
$F2,16 = 5·094$ $P < 0·05$ (among drugs).

Circulating Eosinophile Counts (per mm.³)

Table 2

Subjects	Basis	3 hr. after 25 units (*I.M.*) ACTH	Change (per cent)
(1)	139	6	−95·7
(2)	116	17	−85·3
(3)	94	55	−41·5
(4)	55	6	−89·1
(5)	133	11	−91·7
		Mean	80·7

Table 3

Subjects	Basis	3 hr. after 80 mgm. hydrocortisone	Change (per cent)
(1)	161	28	−82·6
(2)	55	17	−69·1
(3)	44	5	−88·6
(4)	50	5	−90·0
(5)	122	17	−86·1
		Mean	83·3

Table 4

Subjects	Basis	3 hr. after 2 gm. methopyrapone	Change (per cent)
(1)	83	61	−26·5
(2)	111	50	−54·9
(3)	144	138	−4·2
(4)	244	239	−2·0
(5)	67	44	−34·3
		Mean	24·4

Table 5

Subjects	Basis	3 hr. after basis of control group	Change (per cent)
(1)	189	172	−9·0
(2)	117	105	−10·2
(3)	50	61	+22·0
(4)	339	310	−8·5
(5)	144	139	−3·5
		Mean	1·8

in a mean percentage fall of 62·2 in circulating eosinophiles in 3 hr. Hydrocortisone in 80-mgm. dosage also caused a mean fall of 75·6 per cent[4]. The results of the present experiment indicate the similar significant eosinopenic response to the exogenous ACTH and hydrocortisone. An infusion of 1 gm. methopyrapone in 250 ml. 0·9 per cent saline in 2 hr. has been reported to raise 17-hydroxycorticoids in bloods significantly within 4 hr.[5]. The rise in 17-hydroxycorticoids is attributed to uninhibited pituitary secretion stimulating the production of Porter-Silber chromogens.

The eosinopenic response to ACTH and hydrocortisone is uniformly high and without significant difference (Tables 2 and 3). The eosinopenic response to methopyrapone versus control is significant, but low in comparison with that for groups treated with ACTH and hydrocortisone (Tables 4 and 5). Methopyrapone stimulates the secretion of corticotrophin in the pituitary to such an effective level as to cause a significant fall in the circulating eosinophiles.

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Precorticotrophin in Blood

The existence of precorticotrophin, precursor of corticotrophin, has been demonstrated by Dasgupta and Young[1]. Extracts of ox anterior pituitary, otherwise inactive, acquire a corticotrophic activity detectable by Sayers' test (adrenal ascorbic acid depletion) when the medium is acidified (*p*H 3) or when urea (3–8 *M*) is added to it. These findings have been confirmed by Dixon *et al.*[2], who observed the presence of precorticotrophin in pig pituitary also (personal communication). Earlier, Moruzzi *et al.*[3] reported the presence of an 'inactive' form of corticotrophin (activable by treatment with oxycellulose) in the blood of patients suffering from various types of adrenal insufficiency, including Addison's disease; they called it 'activable' corticotrophin. The possibility that 'precorticotrophin' may be comparable with 'activable' corticotrophin and that the former may be in circulation even under normal physiological conditions was discussed by Dasgupta[4]. The present report is concerned with the actual demonstration of precorticotrophin in normal rabbit blood.

When fresh serum obtained from normal rabbit blood is diluted 15–20 fold by adding 0·9 per cent sodium chloride solution no activity is detected when tested by the method of Sayers as modified by Chowdhuri[5]. The modification consists of using morphinized (morphine sulphate 1·5 mgm. per 100 gm. body-weight by subcutaneous route 2–3 hr. prior to administration of test doses) rats as test animals instead of hypophysectomized ones. When, however, the serum is either acidified with 2 *N* hydrochloric acid to *p*H 3 or treated with urea (3 *M*) and then diluted, adrenal ascorbic acid depleting activity is found to be present in the final test solutions (Table 1).

In a second series of experiments, instead of fresh serum, freeze-dried serum was used as the test material. The solid residue left after freeze-drying serum is dissolved in distilled water such that 1 ml. of the solution corresponds to 2 mgm. of the solid. The clear solution is divided into three portions. The first portion is left untreated, the second portion is acidified to *p*H 3 and the third is treated with urea (3 *M*). Each of these three portions is next separately diluted 20-fold with distilled water and its adrenal ascorbic acid depleting activity determined. Whereas no corticotrophic response is produced by the untreated solution, those treated show definite corticotrophic activity (Table 1).

These results provide evidence suggesting the existence in normal blood of a precorticotrophic factor inactive *per se* but giving rise to corticotrophin under the influence of *p*H adjustment (*p*H 3) or urea.

The extent of dilution (15–20 fold) of the serum was determined by trial such that the concentration of endogenous corticotrophin present in the serum was reduced to a very low value and caused no depletion of adrenal ascorbic acid. However, the same serum, despite dilution, was found to maintain the concentration of endogenous precorticotrophin at a sufficiently high level as to give in consequence of *p*H adjustment or treatment with urea a positive response in the Sayers test. The serum, therefore, seemed to be behaving in this respect in a manner analogous to the

Table 1. Mean Response in Sayers' Test produced by Normal Rabbit Serum

	Fresh serum			Freeze-dried serum		
	Untreated (control)	Adjusted to *p*H 3	Treated with urea	Untreated (control)	Adjusted to *p*H 3	Treated with urea
Mean response (ascorbic acid change mgm./100 gm. adrenal) ± Standard error	−4·4 ± 3·34 (20)	−95·7 ± 13·00 (14)	−87·2 ± 8·82 (10)	−0·125 ± 4·86 (8)	−92·0 ± 23·96 (5)	−55·6 ± 16·18 (5)

Figures within parentheses indicate the total number of observations. Test solutions were injected intravenously at a dose of 0·25 ml./100 gm. body-weight.

extracts of ox anterior pituitary prepared by Dasgupta and Young[1], Dixon *et al.*[2] and Dasgupta[4].

Since the freeze-dried serum residue redissolved in distilled water behaved like fresh serum under the same experimental conditions (Table 1), it appears that the precorticotrophic factor of blood survives a single freezing treatment. Moreover, the serum sample was prepared 4–6 weeks before biological testing and this indicates that the circulating precorticotrophic factor is fairly stable in dry conditions.

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PHARMACOLOGY

Augmentation of Toxicity of Monoamine Oxidase Inhibitor by Thyroid Feeding

The administration of desiccated thyroid or thyroxine modifies the susceptibility of animals to the poisonous effects of a number of substances. Thyroid hormone is known to increase the toxicity of fluoride[1], endotoxin[2], alloxan[3], cocaine[4], morphine[5], anoxia[6,7], ozone[8], dinitrophenol[9] and adrenaline[10,11]; furthermore, it prolongs the action of certain barbiturates[12]. Conversely, a diminished sensitivity to the toxicity of a number of nitriles has been reported[13,14].

As a consequence of certain work on subacute thyroid and monoamine oxidase inhibitor interaction, apparent augmentations were observed in the mortality of hyperthyroid rats receiving high daily parenteral doses of a hydrazide (iproniazid) or a non-hydrazide (*N*-methyl-*N*-benzyl-2-propynylamine hydrochloride; '*MO*–911') enzyme inhibitor. The latter drug was first described by Taylor *et al.*[15,16]. To evaluate this possible hormonal sensitization, four groups of fasted male rats (Wistar strain) (172 gm. mean weight), eight animals to a group, were injected intraperitoneally with doses of '*MO*–911' differing by 0·03 log unit. Four other groups (127 gm. mean weight), previously made hyperthyroid by a 7-day desiccated thyroid (2 per cent) *ad lib.* diet, were injected in an identical manner. Nine hyperthyroid rats (130 gm. mean weight) were injected solely with normal physiological saline solution and served as controls. All animals were housed at a temperature of 25–27° C. and were provided prior to monoamine oxidase inhibitor medication with oral vitamin A and D (oleum percomorphum) supplementation at 3-day intervals. The acute toxicity results are given in Table 1.

Table 1. Acute Toxicity of 'MO–911'

Treatment	Dose (mgm./kgm.)	Response (12 hr.)	Dead (per cent)
'MO–911' intraperitoneal	187·5	4/8	50·0
	175·0	2/8	25·0
	163·0	3/8	37·5
	152·4	2/8	25·0
'MO–911' intraperitoneal + thyroid (2 per cent) diet	187·5	8/8	100·0*
	175·0	8/8	100·0*
	163·0	8/8	100·0*
	152·4	8/8	100·0
Thyroid diet controls + 0·14 ml. normal saline intraperitoneal	—	0/9	0

* All deaths within 2 hr. of dosing.

Because a perfect statistical analysis of the respective LD_{50}'s was not possible due to the limited number of animals employed and the one group aberrantly responding to '*MO*–911' at 175 mgm./kgm., only an approximation to this dose for the enzyme inhibitor in the euthyroid animals could be made; this value was found to be 183·5 ± 15·0 (*S.E.*) mgm./kgm. using a graphic method of analysis[17]. It should be noted that in the thyroid-'*MO*–911' treated group all deaths, except one at the 152·4 mgm./kgm. level, occurred within 2 hr. These results show rather conclusively that the acute intraperitoneal toxicity of '*MO*–911' in hyperthyroid rats is markedly increased and that the LD_{50} of the drug lies well below 152 mgm./kgm. in these circumstances.

The cause of this augmented lethality is not easily understood, but because of the early onset of malaise and death, a direct sensitivity to the toxic effects of the monoamine oxidase inhibitor molecule *per se* seems to be the most likely explanation. However, these effects may be possibly related to monoamine oxidase inhibition coupled with the similar enzymatic *in vivo* effects of the thyroid feeding[18,19] resulting in an acute liberation of unbound catecholamines, a diminished ability of the animal to cope with stress and a predisposition to fatal thyrotoxicosis. In any event, this potentiation of toxicity may well explain the reported psychotherapeutic incompatibility of simultaneous medication with an irreversible amine oxidase inhibitor and thyroid extract[20,21].

A more detailed account of these experiments will be published elsewhere.

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High Concentration of (−)-Noradrenaline in *Portulaca oleracea* L.

Portulaca oleracea L. is one of the plants reputed to be of value in the treatment of cardio-vascular diseases in Jamaican folk-lore[1]. It was therefore examined for the possible occurrence of pharmacologically active substances.

A crude protein-free extract was prepared as follows: the fresh plant was macerated in cold 2 *N* hydrochloric acid with a Waring blender. Protein was precipitated by adding ethanol (to approximately 75 per cent) and the supernatant was evaporated at low temperature, under reduced pressure, and in the absence of oxygen to remove the ethanol. The concentrate was diluted with water to an appropriate volume. A strong pressor response was observed when this crude extract was injected intravenously into a dog anæsthetized with nembutal. Because of this response, the possibility of the presence of catechol amines was examined.

The crude extract was examined by paper chromatography using three solvent systems as indicated in Table 1, common catechol amines being used as markers. When the paper was developed with potassium ferricyanide reagent[2], four spots were observed. Three of these spots corresponded to markers of dopa, noradrenaline, and dopamine while the fourth spot is as yet to be identified. The areas of these spots on chromatograms demonstrated approximately equal quantities of noradrenaline and dopamine to be present while the amount of dopa was much smaller.

In order to separate these catechol amines the following procedure was used: the crude protein-free extract was adjusted to *p*H 4 with 10 per cent sodium hydroxide solution and passed through a column of 'Dowex 50–*x*4' (hydrogen form). The catechol anines in the column were eluted with 2 *N* hydrochloric acid. The fraction which contained mainly noradrenaline was subjected to chromatography on sheets of Whatman 3*MM* paper using *n*-butanol/acetic acid/water (4 : 1 : 5) as the solvent system. The appropriate strips from these sheets (located by trial cuttings developed with the ferricyanide reagent) were cut and eluted with 2 *N* hydrochloric acid. The eluates were pooled and were freeze-dried to a brown powder which gave a single spot on the paper in the same position as a marker of noradrenaline. Quantitative comparison of the extracted material with synthetic noradrenaline hydrochloride on paper revealed that equal amounts of each gave comparable spot areas.

Table 1. Paper Chromatography of Catechol Amines of the Crude Extracts of *Portulaca oleracea* L.

System	R_F value						
	Spot I	Dopa	Spot II	Noradrenaline	Spot III	Dopamine	Spot IV
n-Butanol/acetic acid/water (4 : 1 : 5)	0·21	0·21	0·34	0·34	0·41	0·42	0·61
Phenol–water (HCl vapour)	0·16	0·14	0·23	0·23	0·41	0·41	0·54
n-Butanol–0·5 *N* HCl	0·17	0·18	0·12	0·12	0·20	0·20	0·33

The biological activity of the extracted material was then examined. It was found to give a pressor response comparable with that of an equal amount of synthetic (−)-noradrenaline hydrochloride when administered intravenously into dogs anæsthetized with nembutal. This result not only supports the chromatographic evidence for the identity of noradrenaline but also suggests that the noradrenaline present is the biologically active (−)-isomer.

Dopa and dopamine are known to be common plant constituents, but noradrenaline in plants has only been reported previously in banana, plantain, and in potato[3–5]. However, the concentration of noradrenaline found in *Portulaca* is much greater than that in any of these plants. Both by paper chromatography and biological estimation it was found that one of the crude extracts contained noradrenaline approximately equivalent to 2·5 mgm./gm. of fresh plant. It is of interest to note that the concentration of noradrenaline in this plant might be greater than that extractable from suprarenal glands of mammals.

This work forms part of a pharmacological and chemical analysis of some West Indian medicinal plants and is supported by the Tropical Products Institute, Department of Scientific and Industrial Research.

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HISTOCHEMISTRY

Sialic Acid as a Structural Component of Some Mammalian Tissue Cell Surfaces

When human erythrocytes are treated with trypsin, a sialomucopeptide is liberated from their surfaces[1]. It is a common observation that a mucoid material is liberated from mammalian cells by trypsin treatment; the release of this material is associated with a considerable loss of cellular dry mass[2], and alteration of the stickiness of cells for various surfaces *in vitro*[3]. Attempts to investigate the chemical nature of the liberated mucoid[4] have been largely unsuccessful, partly owing to the lack of sensitivity of the tests, and partly because the mucoid cannot be obtained free of cells.

In view of the possibility that the structure of ovine salivary gland mucoprotein put forward by Gottschalk[5] is a general model for the molecular arrangement of mucoprotein at the cell surface, it is important to know whether sialic acid is present at the surfaces of mammalian tissue cells, and if so whether it occurs as a structural element as opposed to a cellular excretion.

A method has recently been developed for measuring the strength of attachment to glass of tissue cells in culture[6]. It consists of growing cells covered by a fluid medium, in circular culture vessels; a rotating disk is lowered into the fluid covering the cells and a shearing stress, transmitted through the

fluid, tends to detach the cells from their glass substratum. With a knowledge of the angular velocity of the rotating disk, the viscosity of the culture medium, the distance separating the bottom of the rotating disk from the cells, and the distance of the cells from the centre of the disk, it is possible to apply accurately reproducible and approximately known detaching pressures to the cells. The cells are counted before and after the application of the shearing stresses, and the percentage of cells detached is determined.

It was found that when a cell is removed from a glass substratum under these conditions, separation is not cleanly at the cell surface/substratum interface, but occurs in the cell surfaces, parts of which are left on the glass[7]. Thus this type of peeling-test provides a measure of the cohesiveness or shear-strength of cell surfaces, rather than measurement of the strength of adhesion between cell and substratum. This differentiation between cohesion and adhesion is in accordance with work done in other fields[8,9].

Recently, an attempt has been made to identify structural components in the cell surface, by incubating cultures with enzymes and determining the resulting change in the shear-strength of the cell surfaces as reflected in the percentage of cells detached by various shear stresses.

For these experiments three 'permanent' strains of cells were used, two (16*R* and 16*C*) obtained from late rat fœtal skin and the other from bovine kidney cortex, and also freshly isolated fibroblasts from rat fœtal skin. It was found that in all these cultures crude receptor-destroying enzyme (Burroughs Wellcome) obtained from *Vibrio choleræ*[10,11] weakened the cohesive strengths of the cellular surfaces. These experiments were not entirely satisfactory as a means of identifying sialic acid, however, since this crude enzyme preparation may also contain a mucinase, a trypsin-like enzyme, and an *N*-acetylneuraminic acid aldolase, as well as 0·01 per cent 'Thiomersal' (sodium ethyl-mercuri-thiosalicylate) preservative.

A very small amount of pure recrystallized neuraminidase[12] was then obtained, which was just sufficient to do one series of confirmatory tests on the 16*R* strain of cells. The cells were grown on glass in synthetic medium 199 (Glaxo) plus 15 per cent bovine serum for 18 hr.; they were washed gently with Hanks's balanced saline solution at *p*H 6·9 and counted in delineated areas. Neuraminidase (10,000 units per ml.) in Hanks's fluid was then applied for 60 min. at 37° C., after which the cells were exposed to a shearing stress of approximately 32 dynes/cm.^{2} for 60 sec. and again counted. 16 observations, each on groups of 200–300 cells, were made on the experimental cultures, and eight on the controls which were incubated with Hanks's fluid only. In the neuraminidase-treated group 37·7 per cent $\pm$ 2·3 (*S.E.*) of cells were detached as opposed to 21·0 per cent $\pm$ 6·0 in the control group. A *t*-test made on the data gave the following results: $t = 3{\cdot}13$, d.f. = 22, $0{\cdot}01 > P > 0{\cdot}001$.

The crystalline neuraminidase used in the present experiments has been very carefully prepared and is very specific in its action[13], which is 'the hydrolytic cleavage of the glycosidic bond joining the keto group of *N*-acetyl-neuraminic acid to D-galactosamine'[14].

It is therefore concluded that sialic acid is an important structural component of the cell surface in one strain, and possibly also in the three other cell types treated with the crude enzyme preparation only.

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HÆMATOLOGY

Immunoelectrophoretic Examination of Agammaglobulinæmia

IT is now well established that congenital agammaglobulinæmia is associated with multiple serum protein deficiencies; the sera of these patients lack 7*S* γ-globulins, 19*S* γ-globulins or β_2M globulin, and β_2A globulin[1]. Good *et al.*[2,3] have recently reported that the sera of patients with agammaglobulinæmia contain three proteins not found in normal human sera and they have termed them β_2K, β_2E and γ_1A globulins; their work was performed with antiserum from a horse immunized against human serum by Dr. P. Grabar. A review of our own immunoelectrophoretic patterns as well as those of Grabar *et al.* has failed to reveal any protein constituent other than C-reactive proteins in the sera of agammaglobulinæmic patients which is not ordinarily detectable in normal sera. Consequently the work reported here was undertaken to re-investigate this matter.

The micromethod of Scheidegger was used for immunoelectrophoresis of sera from normal and agammaglobulinæmic individuals[4]. Electrophoresis was carried out for 3½ hr., at 10 V./cm. in an agar gel containing a borate buffer of 0·1 ionic strength at *p*H 8·6. Following electrophoresis, horse antiserum against whole human serum obtained from Dr. Grabar was placed in a long well cut into the agar to one side of the patient's serum but parallel to the axis of the electrophoresis, and horse antiserum against human γ-globulin prepared by us was placed in an identical well placed on the opposite side of the patient's serum (Fig. 1). Both electrophoresis and the diffusion of antisera into the agar occurred at room temperature. The immunoelectrophoretic patterns were then observed at regular intervals for 48 hr.

Fig. 1 shows that all the bands of precipitation which were obtained with agammaglobulinæmic sera were also obtained with normal sera. The band corresponding to β_2K was elicited with both antisera,

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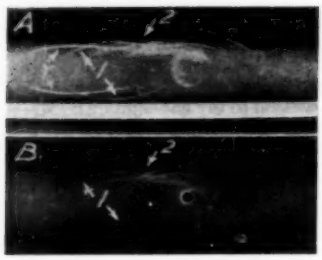

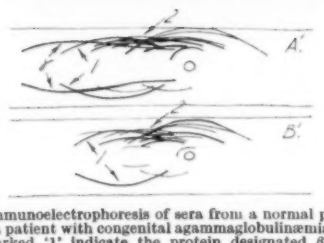

Fig. 1. Immunoelectrophoresis of sera from a normal person (*A*) and from a patient with congenital agammaglobulinæmia (*B*); the arrows marked '1' indicate the protein designated β_2K, those marked '2' indicate that designated β_2E or β_2X and those marked 'γ' indicate the γ-globulins. Figs. *A'* and *B'* are tracings of the precipitation bands seen in Figs. *A* and *B*. Antiserum in the well at upper edge of each figure was horse antiserum versus whole human serum and antiserum in lower well was horse anti-human γ-globulin. Time for diffusion of antiserum was 24 hr.

but the β_2E band was evident only with the antiserum against whole human serum. The identity of the β_2K line formed by each of the two antisera was established by the merging of the ends of these curvilinear precipitation bands. The distance of the vertex of the β_2K band from the electrophoretic axis was the same with both normal and agammaglobulinæmic sera, which suggested that the concentration of the protein component in both sera was similar.

One ml. aliquots of each antiserum were adsorbed with either 0·15 ml. of normal human serum or with 0·15 ml. of a 1:10 dilution of normal human serum. In both instances the adsorbed antisera no longer formed β_2K or β_2E bands with either normal or agammaglobulinæmic sera. These results indicated that normal serum contained β_2K and β_2E globulins in quantities sufficient to remove their respective antibodies from the horse antisera.

The protein which had been designated γ_1A by Good *et al.* had previously been noted by Heremans, who labelled this band of precipitation γ_x-globulin[5]. Heremans noted its occurrence in a variety of pathological states and has shown that it is probably C-reactive protein. The French workers had also noted the band of precipitation designated as β_2E by Good and had variously labelled this band β_2X or β_2B; Heremans stated that it was present in all normal sera which are fresh. A protein corresponding to that called β_2K globulin by Good *et al.* was demonstrated by Scheidegger in the sera of normal persons as well as in the serum of a patient with a dysgammaglobulinæmia characterized by the absence of β_2A and β_2M globulins from the serum. The same horse antiserum against human γ-globulin that we used in the present work had been used in Scheidegger's study[6].

These findings indicate that there is no definite evidence as yet for the presence of abnormal globulins in the sera of patients with agammaglobulinæmia or for the presence in such sera of globulins not demonstrable in normal persons.

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Nature of the Coating Globulin causing a Positive Antiglobulin Consumption Test

The direct antiglobulin consumption test has been developed in several laboratories and has been applied chiefly to the detection of antibodies that sensitize thrombocytes and leucocytes[1–6].

The test is based on the following principle: Pure, thoroughly washed suspensions of leucocytes and thrombocytes obtained from the blood to be examined are added to an antiglobulin serum of known titre and are left at room temperature for 30 min. The supernatant fluid is then tested for its antiglobulin titre against red blood cells coated with incomplete anti-*D* antibodies. If the cells have been sensitized *in vivo*, a significant drop in the antiglobulin titre occurs, while normal cells do not cause any reduction in titre.

The nature of the coating globulin causing a positive antiglobulin consumption test of thrombocytes and leucocytes has not been defined (ref. 6 and Dausset, J., personal communication), although some attempts have been made in this connexion with the globulin coating of red blood cells causing a positive Coombs test[7,8]. In the present work some experiments are reported which suggest that the coating substance causing a direct antiglobulin consumption test of thrombocytes and leucocytes may be a normal plasma protein attaching itself to the cells under certain conditions. The coating substance must not be an antibody produced as a result of an antigenic stimulus.

Preparation of the anti-human globulin serum, the coating of erythrocytes with incomplete anti-*D* antibodies, the anticoagulant and the washing fluid, as well as the separation of the cells from the blood, were performed as described in ref. 6.

The direct antiglobulin consumption test[6] was performed with some minor changes. To two tubes, each containing 0·5 ml. of anti-human globulin serum, 0·1 ml. of repeatedly washed thrombocytes and 0·1 ml. of repeatedly washed leucocytes, respectively, were added and re-suspended in the serum by gentle shaking. The respective blood cell concentration of the samples in the antiglobulin serum was as follows: thrombocytes 50,000–100,000/mm.³, and leucocytes 40,000/mm.³. The tubes were kept at room temperature (20°–25° C.) for 30 min. They were then centrifuged for 10 min. at 3,000 r.p.m. and the supernatants titrated against *O Rh*+ erythrocytes, sensit-

Table 1. Results of Direct Antiglobulin Consumption Test with Leucocytes and Thrombocytes incubated in Autoplasma for Various Times at Different Temperatures

	Separated immediately	After 2 hr.	After 4 hr.	After 6 hr.	After 8 hr.	After 10 hr.	After 12 hr.	After 24 hr.
Leucocytes,								
6° C.	−	−	−	−	±	+	++	+++
25 °C.	−	−	±	±	++	+++	+++	+++
37° C.	−	−	±	++	+++	+++	+++	+++
Thrombocytes,								
6° C.	−	−	−	−	±	±	++	+++
25° C.	−	−	+	+	++	+++	+++	+++
37° C.	−	±	++	++	+++	+++	+++	+++

+++, Strong antiglobulin consumption, that is, positive test.
++, +, ±, Degrees of antiglobulin consumption.
−, No consumption, that is, negative test.

ized with incomplete anti-*Rh* antibodies. The titre was expressed in terms of the last tube showing macroscopical agglutination.

Whole blood taken into siliconized syringes containing disodium sequestrene as anticoagulant in a proportion of 1 : 9 was divided into aliquots of equal amounts. From the first the thrombocytes and leucocytes were separated immediately and washed three times. The second sample was kept for various times at either 6° C., 25° C. or 37° C. before the cells were separated and washed. The direct antiglobulin consumption test was then performed at the same time on the cells which had been separated at once from the blood and on those that were incubated before separation. Results of such experiments are summarized in Table 1. It will be seen that a direct relationship could be demonstrated between the temperature, time of incubation and the degree of the antiglobulin consumption. If the cells were immediately separated from the blood after its withdrawal, the antiglobulin test was always negative, while the test became positive after keeping the blood for 4–6 hr. at 37° C. or 8–10 hr. at room temperature and more than 10 hr. in the refrigerator.

Table 1 gives typical results of one such experiment. Twenty blood samples from normal healthy donors were examined and all of them showed similar results, although the degree of the globulin coating *in vitro* of the thrombocytes and leucocytes varied significantly from sample to sample.

The results reported indicate that leucocytes and thrombocytes may absorb *in vitro* plasma proteins, which adhere to the cells very strongly, so that even repeated washings do not separate them. The absorbed protein causes a positive direct antiglobulin consumption test, indistinguishable from the test obtained when cells are sensitized *in vivo* due, presumably, to an immune mechanism.

It is tempting to believe that the globulin coating of the leucocytes and thrombocytes *in vitro* was achieved as a result of some damage to the cell in its abnormal surroundings. The fact that at 37° C. the sensitization by autoplasma proteins was quicker than at 6° C. points to the same conclusion.

If the results obtained *in vitro* may be compared with *in vivo* processes, we may assume that bacterial toxins, breakdown products of drugs, high temperature or any other damage to the cells in the body may change them in such a way as to prepare them to absorb irreversibly normal autoplasma proteins.

The finding, therefore, of a positive antiglobulin consumption test indicates only that the cells are coated with globulin, which may either be an antibody or a normal protein absorbed on a damaged cell.

The clinical significance of the coating as a result of an antibody or the coating as a result of the interaction of the cells with normal autoplasma proteins is not yet clear, although it may be very similar.

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PATHOLOGY

Development in Mice of Prolonged Non-specific Resistance to Sarcoma Implant and Staphylococcus Infection following Repository Injection of Lipopolysaccharide

Previous reports have indicated that the injection of lipopolysaccharides (endotoxin) of Gram-negative organisms exert a profound effect on many vital functions of an animal[1,2]. Particularly striking has been the development of a non-specific resistance against infection[2,5]. More recently, resistance against implantation of malignant tumours has also been demonstrated following treatment with agents stimulating the reticulo-endothelial system, including lipopolysaccharides[6,7]. However, use of lipopolysaccharides clinically has, so far, been frustrated in view of the hazards of fatal shock and/or infection, since the action of the endotoxin is biphasic[4]. For several hours following the injection of the lipopolysaccharide, resistance to infection is markedly decreased, but if the animal survives the shock and other physiological derangements, the resistance to infection is increased manyfold. The administration of lipopolysaccharide is particularly hazardous in experimental animals with latent or low-grade infections, converting these into acute processes often terminating in death[8].

To overcome the hazards of shock and the decreased resistance developed during the negative phase, which is an expression of excess dosage or limited tolerance, it was thought advisable to administer the lipopolysaccharide in repository form. Prepared as a water-in-oil emulsion in which mineral oil is the outer phase, the endotoxin is released slowly owing to the poor metabolism of the oil.

It is the purpose of this communication to summarize the results of experiments with emulsified lipopolysaccharide, in which single large doses ($2–4 \times LD$ 50) were injected into mice, with the survivors being challenged afterwards in small groups at varying intervals, either by a sarcoma 180 implant, or by an intravenous injection of a virulent staphylococcus. Tables 1 and 2 show the number of animals originally injected with lipopolysaccharide and the number that survived; the number of days post-injection when challenged; the number of survivors for each group; controls (mice receiving the same challenge but pre-treated only with saline); and the increases in the size of the liver and spleen of the endotoxin-treated animals as compared with

Table 1. Survival of Mice following Implantation of Sarcoma-180 after Priming with a Single Injection of Emulsified Endotoxin*

Day of challenge post-injection of endotoxin	No. of animals and material injected	No. of survivors	Weight of organs (mgm.)† Spleen	Liver
2	6 Endotoxin	2	305	1,175
	6 Saline	0	306	1,180
13	5 Endotoxin	2	336	1,710
	5 Saline	0	313	1,550
30	10 Endotoxin	8	528	2,059
	10 Saline	2	370	1,804
44	10 Endotoxin	5	650	2,151
	10 Saline	2	412	1,850
61	10 Endotoxin	6	720	2,320
	10 Saline	2	423	1,872
92	17 Endotoxin	5	745	2,410
	10 Saline	1	525	1,976

* *E. coli* lipopolysaccharide *O26* (*B6* Difco). 110 mice injected with 2 mgm./0·1 ml. of emulsion—67 survived.
† One of each of the treated and control groups was killed before challenge.

the controls. One of each group was killed on the varying days of challenge for these measurements, without challenge by either implant or infection.

Analysis of Tables 1 and 2 shows the following: The intramuscular injection of emulsified endotoxin in 1-mgm. doses resulted in the death of only 5 out of 135 animals. On the other hand, 2-mgm. doses resulted in the loss of 43 of 110 mice injected initially. Deaths here, however, cannot be attributed solely to the dosage since on other occasions the same dosage of better emulsions (droplet size 1–2μ) did not kill. The emulsion employed in this instance had shown droplets of about 4μ, which may have contributed to the higher number of deaths.

Resistance against implantation of the sarcoma becomes evident in the pre-treated animal when challenged on the second day post-injection, reaching a peak about the 30th day, and maintaining this resistance as long as 92 days (Table 1). Resistance against the *Staphylococcus* challenge was more striking since 114 of 115 control animals died, whereas the survival-rate in the sarcoma implantation was 20 per cent in 3 control groups. The highest peak of resistance (80 per cent) was noted against sarcoma implant, whereas the resistance against the *Staphylococcus* reached a peak of 60 per cent. However, when consideration is given to the dose of lipopolysaccharide administered and the survival-rate of the control animals, the resistance against the infection becomes most significant.

The enlargement of both liver and spleen following a single repository injection is noteworthy, since the development of non-specific resistance is asserted to be due to the stimulation of the reticulo-endothelial system[3]. These experiments show, however, that resistance is displayed long before there is maximum enlargement of these organs.

Table 2. Survival of Mice following an Intravenous Injection of Hemolytic *Staphylococcus* after Priming with a Single Injection of Emulsified Endotoxin*

Day of challenge post-injection of endotoxin	No. of animals and material injected	No. of survivors	Weight of organs (mgm.)† Spleen	Liver
8	10 Endotoxin	6	306	1,132
	10 Saline	0	300	1,062
14	10 Endotoxin	4	490	1,192
	10 Saline	0	401	1,094
26	20 Endotoxin	12	540	1,590
	20 Saline	0	420	1,261
40	20 Endotoxin	10	601	1,622
	20 Saline	1	463	1,321
55	20 Endotoxin	8	632	1,810
	20 Saline	0	451	1,681
78	15 Endotoxin	1	459	1,960
	15 Saline	0	450	1,875
90	20 Endotoxin	0	467	1,910
	20 Saline	0	455	1,891

* *E. coli* lipopolysaccharide *O26* (*B6* Difco). 135 mice injected with 1 mgm./0·1 ml. of emulsion—130 survived.
† One of each of the treated and control groups was killed before challenge.

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The Effect of Various Diets upon Sexual Maturity of Mice

It was demonstrated previously that eggs are carcinogenic in mice[1]. On the other hand, it was found that chicks maintained on an egg diet become sexually mature and the hens begin to lay eggs 6–8 weeks earlier than the controls (chicks of the same breed maintained on a Purina mash diet[2]). The question was consequently raised whether these two phenomena, carcinogenicity and sexual precocity, are caused by a single, or by two different components of the egg. It was important, therefore, to determine the effect of egg-yolk and egg-white upon sexual maturity of mice and compare it with the carcinogenic effect.

Mice of the *T.M.* strain were used in the present experiments. This is an inbred strain of mice developed in the School of Tropical Medicine, and in the 13 years of its existence no malignancies were observed. On a diet supplemented with eggs, however, the mice of this strain developed as high an incidence of cancer as the *A* strain[3]. The mice were divided into 4 groups and at the age of 4 weeks they were placed on the following diets. Group 1, the control, was maintained on the Rockland rat diet, no supplement. For the other 3 groups the Rockland rat diet was supplemented with raw egg-yolk (group 2), hard-boiled egg-white (group 3), or a mixture of lard, cholesterol and sugar (group 4). One egg-yolk, one egg-white or half a gm. of lard mixed with 50–100 mgm. of cholesterol and with half a gm. of sugar were given daily per cage containing 8 mice. The mice of the 4 groups were bred and their offsprings, which were maintained on the same diets, were used in the present experiments.

The age of the mice at the time they gave birth to the first litter varied considerably. As shown in Table 1, the mice of the second group, on the Rockland rat diet supplemented with raw egg-yolk, were the most precocious. They gave birth to the first litter at the average age of 66 days, or 8 days earlier than the controls. On the contrary, the mice on the diet supplemented with egg-white were delayed in their sexual development; they first reproduced at the average age of 94 days, or 21 days later than the controls. These differences between the two experimental groups and the controls appear to be highly significant. The mice of the fourth group, on the diet supplemented with lard, cholesterol and sugar, first reproduced at the average age of 75·4 days, or one

Table 1

	Diets	Degrees of freedom	Mean ± S.E.M.*	Variance	Pooled variance (control and experimental)	Standard error of difference between means	Difference between control and experimental means	t	P
1	Rockland rat diet	40	73·5 ± 8·3	68·96	—	—	—	—	—
2	Rockland rat diet + yolk	44	65·8 ± 5·6	31·47	49·32	1·5	7·7	5·13	< 0·1%
3	Rockland rat diet + white	43	94·5 ± 20·5	422·26	251·99	3·4	21·0	6·18	< 0·1%
4	Rockland rat diet + lard and cholesterol	44	74·6 ± 8·4	70·56	69·80	1·8	1·1	0·61	> 20·0%

* S.E.M. $= \sqrt{\frac{(X - \bar{X})^2}{n - 1}}$

day later than the controls. This difference, however, does not seem to be statistically significant.

In summary, the yolk of eggs accelerates sexual development, while the white slows it down. Since preliminary results of other experiments indicate that not only the egg-yolk but also the egg-white is carcinogenic, the conclusion can be drawn that the carcinogenic and sex-stimulating substances in eggs are not the same. There is, however, the possibility that a single substance, present in both the egg-white and egg-yolk, is carcinogenic and stimulates sex development, but a second factor present in the egg-white inhibits sexual maturity thus masking the latter effect. Recently, work has been started with alcohol and ether extracts of whole eggs and of yolk. Some of the mice on the alcohol extract have already died of cancer. The same extract, however, does not seem to cause precocious sexual development, which indicates that our first assumption is correct; namely, that there are two different substances in eggs, one carcinogenic and the other one stimulating sex development.

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Effect of Alpha-Amylase on the Ehrlich Mouse Ascites Tumour

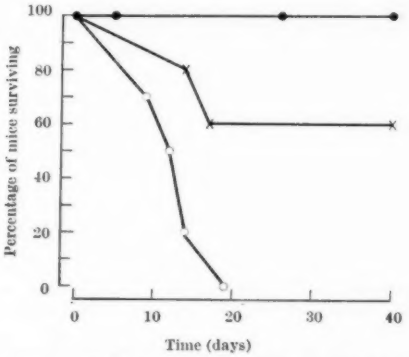
Even after incubation in saline at 37° for as long as 24 hr., about $8·5 \times 10^6$ Ehrlich ascites tumour cells will produce tumour and cause death in 20–26 days when re-injected into new hosts. As part of an attempt to pin-point *in vitro* specific types of damage and then to test the virulence of these damaged cells by subsequent inoculation into mice, Ehrlich ascites tumour cells were exposed to the action of the enzyme α-amylase.

A number of white Swiss mice were inoculated intraperitoneally with 0·3 c.c. of fresh ascitic fluid. After seven days, the ascitic fluid was withdrawn, centrifuged for 10 min. at 4,000 rev./min. and then washed three times with triple volumes of 0·85 per cent saline. 1 c.c. of these preparations of packed tumour cells contained about 34×10^7 cells (counted in a routine hæmocytometer and diluted in 0·1 per cent citric acid). The packed cells were suspended in twice their volume of saline and incubated at 37° in a water-bath equipped with a shaker following the addition of 25 mgm. of α-amylase (three times recrystallized, Worthington) in 0·5 c.c. water for each 34×10^7 cells. The control cell flasks received 0·5 c.c. distilled water; 500 units of penicillin per c.c. of incubation mixture were always added. After 3 and 7 hr. respectively, the cell suspensions were diluted with saline, so that 1 c.c. contained $8·5 \times 10^6$ cells. This amount was injected intraperitoneally into 15 white Swiss mice (weight 20–22 gm.) and their survival-times were followed.

A representative experiment is shown in Fig. 1. Six such experiments were carried out. Mice injected with the 0-, 3- and 7-hr. control mixture showed approximately the same survival-rates, apparently because of the large inoculum of tumour cells (at 0 hr. about 1/10 of this amount would have sufficed for production of ascitic tumour). All mice died of ascites tumour within 20 days after inoculation and 50 per cent mortality occurred on about the twelfth day. Mice injected with cell preparations incubated with α-amylase for 3 hr. showed a 40 per cent tumour mortality within 40 days. Increasing the incubation time with enzyme to 7 hr. resulted in a further loss of virulence, since none of the mice inoculated with these preparations developed an ascitic tumour within 40 days. β-amylase was found to have no effect on the virulence of these tumour cells.

Cell counts carried out at the beginning and end of the incubation showed no appreciable change in number of cells.

DNA and RNA were determined at 0 hr. and after 7 hr. incubation by Schmidt and Thannhauser's method[1]. The DNA phosphorus ranged from 1·6 to $2·2 \times 10^{-9}$ mgm./cell and the RNA phosphorus from 3·5 to $4·9 \times 10^{-9}$ mgm./cell in different preparations. No appreciable decrease in the tumour cell DNA and RNA content was noted in any experiment.



Percentage survival of mice inoculated with the following cell mixtures. ○—○, Untreated cells incubated for 7 hr.; ×—×, cells incubated with α-amylase for 3 hr.; ●—●, cells incubated with α-amylase for 7 hr.

Cytological preparation from representative incubation mixtures were stained by the Papanicolaou technique and examined without knowledge as to the treatment previously given. Depending on the appearance of their nuclei, cells were considered to be either viable or non-viable. There was no appreciable difference in the proportion of non-viable cells between preparations made from the 0-hr. control mixture and those made after 3 hr. incubation, either with or without α-amylase. There was, however, a marked increase in the ratio of non-viable to viable cells both in the treated and untreated mixtures after 7 hr. incubation. The difference between the two groups at 7 hr. was slight ; about 30 per cent of the control preparations appeared to have remained viable compared with about 20 per cent of the cell suspensions treated with amylase.

Because of the frequent assumption that viable cells are resistant to eosin, all these cell suspensions were examined by Schrek's method[2]. The 0-hr. preparations contained 93–95 per cent, the 7-hr. control preparation about 80–90 per cent and the 7-hr. preparations incubated in the presence of α-amylase about 80–95 per cent resistant to eosin cells. No appreciable amount of material giving a positive reaction with periodic acid–Schiff reagent was found in cell smears from any of the preparations.

Regardless of the unaltered DNA and RNA contents or the lack of apparent cytological distinctions between treated and untreated cells, the tumour cells were impaired in their growth ability after incubation with enzyme for 3 or 7 hr., even after they were returned to an optimal nutritional milieu.

These experiments suggest that a polysaccharide containing 1,4 glycosidic linkages may be important for the virulence of Ehrlich ascites tumour cells.

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Evidence that the L-Asparaginase Activity of Guinea Pig Serum is responsible for its Antilymphoma Effects

The inhibitory action of guinea pig serum on the cells of three transplantable mouse and rat lymphomas *in vivo* was described some years ago in papers from this Laboratory[1,2]. In a continuation of this work, evidence has now been found that the L-asparaginase activity of guinea pig serum is responsible for the antilymphoma effect. The evidence has been obtained in two ways: first, by a study of the induction of resistance to guinea pig serum by 6*C3HED* cells grown in tissue culture; and secondly, by a direct comparison of L-asparaginase activity in various preparations of guinea pig serum with their tumour inhibitory properties.

It was found that 6*C3HED* cells obtained from ascites tumours, and shown to be sensitive to inhibition by guinea pig serum, could be cultivated relatively simply in Eagle's medium[3], which contains L-glutamine, but no L-asparagine. For an initial period of 2–4 weeks growth of the cultured cells was poor, as indicated by a low rate of acid production and diminished cell counts, but with adaptation to the medium the cultures became considerably more vigorous. When, however, cells from the 9 cultures examined were removed after 2–4 weeks incubation and implanted into *ZBC* mice the tumours produced all showed complete resistance to guinea pig serum. Furthermore, the tumour cells having once acquired resistance maintained this character permanently both in prolonged culture and in serial transplantation through *ZBC* hosts. In these features, and particularly in the complete degree of resistance to guinea pig serum, the cultured cells strongly resembled resistant sublines of 6*C3HED* cells obtained by Kidd and Holmquist from tumours treated *in vivo* with small quantities of guinea pig serum[4].

However, the presence of L-asparagine in the medium profoundly modified the characteristics of the culture. In four separate experiments cultures were prepared in parallel using Eagle's medium and a modification containing 200 mgm./l. of both L-glutamine and L-asparagine. The cultures grown in Eagle's medium behaved in the manner already described, initially they grew poorly and when tumour cells were tested after 3 weeks in culture they proved to be fully resistant to guinea pig serum. But in the modified medium growth was more vigorous from the beginning. Cells from the four cultures have been implanted into mice after periods of 12, 11, 9 and 9 weeks incubation respectively. They remain fully sensitive to supression by guinea pig serum.

Modifications of Eagle's medium were also prepared to contain L-aspartic acid, L-alanine, L-arginine, glycine, L-histidine, L-leucine, L-lysine, L-methionine, L-phenylalanine, proline, and L-tyrosine at concentrations of 200 mgm./l. But in these, cultures showed the same growth lag as observed in the original medium and the development of guinea pig serum resistance was completely uninfluenced.

These experiments therefore show the intimate relationship between the metabolic changes induced in 6*C3HED* cells by the absence of an external source of L-asparagine, and the development of resistance to guinea pig serum.

Now guinea pig serum is a particularly rich source of L-asparaginase activity, as was shown by Clementi[5], but activity was not detected in the sera of numerous other species examined. Similarly, Kidd[1] found that antilymphoma activity was confined to guinea pig serum. A comparison of these two activities in preparations derived from guinea pig serum showed a striking correspondence.

Electrophoresis of guinea pig serum by a modification of the starch-block technique of Kunkel and Slater[6] showed that L-asparaginase activity was confined to the α_2 globulin fraction. When eluates from the starch block were injected into groups of 3 *ZBC* mice immediately following the subcutaneous implantation of 5×10^4 6*C3HED* cells of the original guinea pig serum sensitive line into each groin, a marked delay in the appearance of tumours occurred in animals receiving fractions which possessed L-asparaginase activity. No other fraction produced any tumour inhibition. Thus, from the fractionation shown in Fig. 1, eluates in tubes numbered 27–30 caused a delay in the first tumour appearance of 3, 5, 7, and 4 days respectively, compared with untreated controls and animals receiving other eluates, in which tumours first appeared in all groups by the

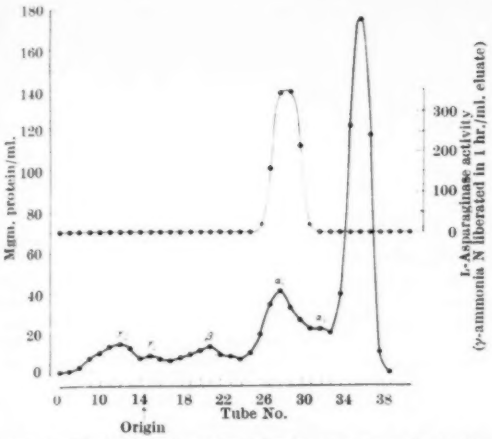

Fig. 1. Electrophoretic pattern of guinea pig serum (in 0·1 *M* veronal buffer, *p*H 8·5) with the localization of L-asparaginase activity

11th day following implantation. Fractions possessing L-asparaginase activity produced complete suppression of many tumours and others appeared at irregular intervals after considerable delay.

Further experiments were performed to examine the stability of L-asparaginase activity in samples of guinea pig serum to changes in temperature and *p*H. Assays of residual enzyme activity provided an accurate correlation with the effectiveness of these samples in producing tumour inhibition.

Ainis *et al.*[7], using the Murphy-Sturm lymphosarcoma, showed that serum from new-born guinea pigs possesses only very weak antilymphoma activity. This finding was confirmed using lymphoma 6*C3HED*. Assays of neonatal serum showed that L-asparaginase activity was only present at 3–15 per cent of adult values.

These experiments provide strong evidence that the inhibitory action of guinea pig serum on 6*C3HED* cells is due to its L-asparaginase activity. It is probable that the antilymphoma action on other sensitive cell strains is by a similar mechanism, and indicates that a distinct form of L-asparagine metabolism is possessed by this group of tumours.

These findings will be published in detail elsewhere. Further experiments are in progress to prepare more highly purified enzyme preparations and to examine the function of L-asparagine in tumour cell metabolism.

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BIOLOGY

Excretion of Inulin by Malpighian Tubules

In an earlier investigation[1] it has been shown by the use of isotopically labelled compounds that the wall of the Malpighian tubule of the stick insect is permeable to urea and to certain sugars and amino-acids. At that time labelled inulin was not available.

Five Malpighian tubules of the stick insect *Dixippus morosus* were isolated in drops of medium containing one part of stick insect Ringer, one part of stick insect serum, a small quantity of the antibiotic oxytetracyclene and inulin labelled with carbon-14 at a concentration of about 0·2 per cent. Urine was collected as described elsewhere[1]. The count-rates of equal volumes of urine and medium were compared (Table 1).

Table 1

Tubule	Urine (*U*) (c.p.m.)	Medium (*P*) (c.p.m.)	*U*/*P*
1	406	8,679	0·047
2	331	8,828	0·038
3	363	9,165	0·040
4	431	9,984	0·043
5	582	9,752	0·060
			Mean = 0·046

In subsequent experiments urine and medium were examined by paper chromatography for evidence of metabolization of inulin; no evidence was found.

The *U*/*P* ratio for inulin may be compared with the *U*/*P* ratios for other carbohydrates: glucose, 0·75; fructose, 0·58; sucrose, 0·58 (ref. 1). On the theory that soluble constituents of the medium enter the urine by diffusion through the wall of the Malpighian tubule[1], this much lower *U*/*P* ratio for inulin is understandable in view of its much greater molecular weight.

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Composition of the Skin Follicle Population in Seven Rajasthan Sheep Breeds

Early workers found that the wool follicles of domestic sheep are mainly arranged in groups of three primaries with their associated secondaries[1]. The existence of a number of groups with only two or one primary (*P*) follicles was also recognized. The proportion of these 'incomplete' trio groups was considered to be so small as to make a negligible difference in the primary lateral/primary central (*PL*/*PC*) ratio. Ross[2] found a *PL*/*PC* ratio of 1·9, hence most workers have assumed the *PL*/*PC* ratio to be equal to 2.

Counts of trio, couplet, and solitary groups, and the number of associated secondary (*S*) follicles were made at superficial levels on whole sections of biopsy skin samples (taken with a trephine, 1 cm. in diameter) of about two-year-old ewes, of seven Rajasthan breeds. The results are given in Table 1.

Table 1. Percentages of Different Follicle Groups, S/P and PL/PC Follicle Ratios, at Mid-side of Rajasthan Sheep Breeds

Breed	No. of sheep	Percentage of total number of groups				Mean S/P ratio					PL/PC
		More than 3	Trio	Couplet	Solitary	More than 3	Trio	Couplet	Solitary	All follicles	
Chokla	7	1·8	64·0	12·6	21·6	2·02	2·54	2·65	2·99	2·58	1·41
Magra	7	0·2	60·2	15·5	24·1	2·75	2·26	1·98	2·49	2·28	1·36
Nali	7	2·7	62·8	13·9	20·6	1·22	2·58	2·57	3·00	2·55	1·45
Jaisalmere	8	0·2	64·3	17·7	17·8	3·87	2·46	2·40	3·36	2·53	1·46
Marware	6	0·4	63·1	12·9	23·6	2·25	2·12	2·14	2·66	2·19	1·39
Malpura	7	0·3	46·3	19·6	33·8	1·75	1·78	1·57	1·78	1·74	1·12
Sonadi	8	0·2	37·3	23·6	38·9	1·00	1·14	1·01	1·19	1·13	0·98

The ratio of PL to PC follicles varies from 0·98 to 1·41 in these Indian breeds and is consistently lower than the figure 1·9 observed by Ross[2] in New Zealand Romney N-type sheep. Further, the proportions of trio groups, which varies from 37 to 64 per cent, is much lower than the figures of more than 90 per cent for Romney N-type sheep (Ross[2], quoted by Fraser and Short[3]).

In the hairy group (Malpura and Sonadi) the PL/PC ratio, the mean S/P ratios and percentage of trio groups is lower and of couplet and solitary groups is higher than in the carpet wool group (Chokla, Magra, Nali, Jaisalmere and Marware).

The trio percentage is higher in the carpet wool group and lower in the hairy wool group than the total of couplet and solitary groups. In these hairy breeds, the trio arrangement is apparently not a predominant feature of the grouping of the P follicles.

As adult maturity is approaching, it becomes progressively more difficult to establish the essential arrangement of the primary follicles in their original trio, couplet or solitary configuration. Hence these results need to be corroborated by qualitative and quantitative histological examination of appropriate pre-natal material, from the different breeds, covering the transition from the pre-trio to the post-trio stage in development of follicle population.

In general, the mean S/P ratio of the solitary groups is greater than that of both couplet and trio groups. Differences in S/P ratio between trio and couplet groups are usually small compared with the differences between solitary and trio or couplet groups.

The mean follicle densities of Sonadi (lowest among these breeds) are compared with those of Chokla (highest) in Table 2.

The PC density can be taken as indicating the density of follicle groups, and hence it is seen that the increase in total follicle density from Sonadi to Chokla is associated with substantial increases in both PL follicles (57 per cent) and S follicles (228 per cent) per group, but a much smaller (14 per cent) increase in the density of the groups themselves. The total population of both P and S follicles has also increased from Sonadi to Chokla, with only a very small increase (7 per cent) in the total number of follicle groups.

Stephenson[4] speculated that "central primary anlagen, once present, may inhibit the initiation of new central primaries within a certain radius". The initiation of new PC follicles may possibly depend on skin expansion taking place simultaneously at the required rate. Since PC follicles directly influence the initiation of PL follicles, for a given rate of skin expansion, the rate of formation of P follicles may vary from different positions, and such variation may also occur between different fœtuses. It may be that variations in formation of P follicle are greater for lateral than for central primaries.

Table 2

	PC	PL	S	$PC + PL + S$
Chokla	1·6	2·2	9·5	13·3
Sonadi	1·4	1·4	2·9	5·7
Sonadi adjusted to body-weight of Chokla	1·5	1·5	3·2	6·2

Two probably independent mechanisms seem to influence the number of S follicles per P: one which provides for an increase of S follicles in those breeds with higher PL densities and another which allows the initiation and development of more S follicles around couplet and solitary primaries.

Helpful comments from Mr. H. B. Carter, of the Animal Breeding Research Organization, Edinburgh, Dr. A. G. Lyne and Dr. B. F. Short, of the Commonwealth Scientific and Industrial Research Organization, Australia, and Mr. N. L. Narayan, of the Animal Husbandry Department of Rajasthan, are acknowledged with thanks.

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Sex Composition of Lamprey Populations

Since 1947 records have been kept of the size and sex composition of annual spawning populations of *Lampetra planeri* in the River Yeo in Somerset. In a previous communication[1] it was reported that up to 1954 there had been marked variations in the sex ratio from year to year and that in general these changes were associated with fluctuations in the relative abundance of this small isolated population. Observations made in subsequent years have confirmed the existence of a positive correlation between abundance and the ratio of males to females; that is, the bigger the population the larger has been the proportion of males. This trend is emphasized in Fig. 1, where the total annual catches have been plotted against the number of males and females separately and the corresponding regression lines fitted to the observations. The lowest observations, which lie close to the intersection of the two regression lines, give sex ratios of 1·2 ♂/♀ for the smallest populations of 50–60, while the highest observed sex ratio of 3·4 corresponds to a relative population of about 200.

In the course of the work on the landlocked sea lamprey (*Petromyzon marinus*) in the Lake Huron-Michigan watersheds, undertaken by the U.S. Depart-

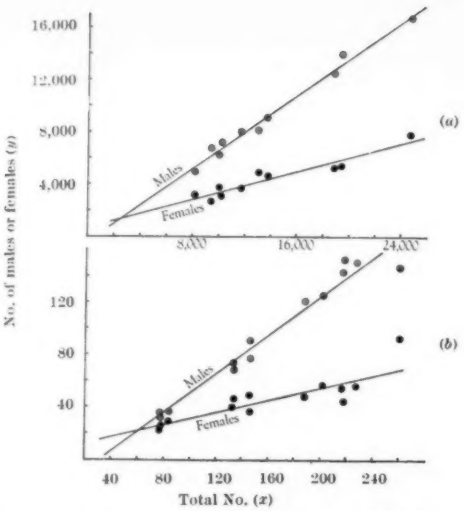

Fig. 1. Changes in the sex ratio of lamprey populations with relative abundance. *a*, *P. marinus* (Oqueoc River, 1944–57): males, $y_{\delta} = -468 + 0{\cdot}71x$; females, $y_{\varphi} = 632 + 0{\cdot}27x$. *b*, *L. planeri* (R. Yeo, 1947–60): males, $y_{\delta} = -9{\cdot}0 + 0{\cdot}74x$; females, $y_{\varphi} = 10{\cdot}5 + 0{\cdot}25x$

ment of the Interior, Fish and Wildlife Service, a somewhat similar situation has come to light in the upstream-migrants of this parasitic species. The data on relative abundance and sex composition for the Oqueoc River, supplied by Dr. V. C. Applegate, have been plotted in the same way as the observations on *L. planeri* (Fig. 1) and the corresponding regression coefficients calculated. The similarity in the curves is indeed remarkable; for the male *planeri* the slope of the regression line as given by the regression coefficient *b* is 0·74 compared with 0·71 for *P. marinus*, while the corresponding values for the females are 0·25 and 0·27 respectively. The correspondence is the more striking when it is borne in mind that, whereas the observations on *P. marinus* are based on very large numbers (8,000–24,000), the information on the brook lamprey populations has been limited to annual catches of 50–200.

In view of this correspondence, it is tempting to search for a common explanation for these changes in the sex composition of the spawning populations. However, it is important to recognize that the pattern of changes in relative abundance have been somewhat different in the two species. In the brook lampreys of the Yeo, there have been no continuous upward or downward trends in population size, but rather a series of annual fluctuations with a distinct tendency for large and small populations to follow one another. In the Great Lakes, on the other hand, there appears to have been a continuous increase in the lamprey population during 1944–49 and during this period the relative abundance increased from an estimated 3,300 in 1944, to 24,600 in 1949. In this phase, the sex ratio also increased from 1·65 ♂/♀ in 1947 to 2·13 in 1949. The highest sex ratios were recorded from 1950–52 (2·56—2·58 ♂/♀) but in subsequent years the excess of males tended to decrease and in 1957 the sex ratio was 1·56, that is, slightly lower than in 1947, when records were first made. Although there have been fluctuations in relative abundance, the general trend of the population during the period 1950–57 has been downwards.

Such a close parallel in the sex composition of spawning populations is the more surprising in view of the great divergence in the life-cycle of the two species. Whereas in *L. planeri* spawning takes place within about six months of metamorphosis, in *P. marinus* a period of parasitic life in the Great Lakes, of about two years duration[2], is interposed between metamorphosis and spawning. The existence of a similar relationship between sex ratio and population size, in spite of geographical differences and diversity of life-cycle, suggests that whatever factors may be responsible, their operation should be sought in those phases of the life-cycle which are common to both species. Moreover, since any attempt to account for this relationship must start from the assumption that the sexes are present in approximately equal proportions during the larval stages[3], this would appear to limit the causal period to the critical stage of metamorphosis or to the period of sexual maturity.

The observed variations in the sex ratio could conceivably be explained by assuming that the mortality-rate is dependent on density and higher in the female lamprey than in the male, but while this would be plausible in the large parasitic populations of *P. marinus*, it is unlikely that it could apply to the small Yeo population of brook lampreys which do not feed in the adult state. Moreover, in view of the differences in environment and size of the two forms, similar patterns of mortality due to predation could almost certainly be excluded.

In a detailed study of the brook lamprey population of the River Yeo, which will be published elsewhere, it has been suggested that the spawning class consists of more than one age group and that fluctuations in abundance may be due to differences in the annual recruitment of these age groups. If this hypothesis is extended to include a differential recruitment of the two sexes with respect to their age class, it is possible to explain the sequences of high and low populations and high and low sex ratios which have been observed in brook lamprey populations during 1947–60. A similar situation, it is thought, might exist in the landlocked sea lamprey, a possibility which could be verified by determining the distribution of the sexes in large numbers of the downstream migrants.

I should like to acknowledge with gratitude the help received from Dr. V. C. Applegate of the U.S. Department of the Interior, Fish and Wildlife Service, both in supplying specimens and in furnishing the information on the sea lamprey.

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Isolation of *Plasmodium knowlesi* from Philippine Macaques

Between October 1959 and November 1960 blood films were examined for parasites from 164 Philippine long-tailed macaques (*Macaca irus*) at the University of California Medical Center. Ten of the animals were found infected with species of *Plasmodium*. Morphological investigations suggested that several species

were present either as single infections or, in several monkeys, as multiple infections. Sub-inoculations into uninfected monkeys were used to help determine the species. Blood from two animals (*MK* 116 and *MK* 119, originally from the Cebu area of the central Philippines) was also sent to the Laboratory of Parasite Chemotherapy in Memphis. This communication has been prepared primarily to record the isolation of *Plasmodium knowlesi* from one of the Philippine macaques. This finding represents a considerable extension of the known range of this parasite which is transmissible to man experimentally and perhaps also in Nature.

On November 15, 1960, 2 ml. of infected blood (scanty plasmodia on thick films) from *M. irus*, *MK* 119, were injected intraperitoneally into an uninfected immature rhesus (*M. mulatta R* 614). Daily blood films from *R* 614 remained negative, and after three weeks it appeared that the transfer had been unsuccessful. On December 5, 1960, *R* 614 was sub-inoculated by intraperitoneal injection of 2 ml. of blood, this time from *M. irus MK* 116. Again the blood was only lightly infected with plasmodia. On the following day a patent plasmodial infection appeared in *R* 614 (5 parasites/1,000 red cells). It later became clear (see later) that this infection was caused by parasites inoculated from *MK* 119 three weeks earlier. The infection evidently became patent as the result of the stimulus due to foreign protein of the second injection of *M. irus* blood.

From December 6 until the work was terminated, blood films were made from *R* 614 every four hours. During a nine-day period beginning on the first post-inoculation day, the plasmodia followed a well-defined 48-hr. asexual cycle with maturation of schizonts being a maximum in the early morning hours, between 1 a.m. and 5 a.m. The blood parasites during this phase of the infection were characteristic of *Plasmodium cynomolgi*, a parasite which has been recorded from Philippine macaques on at least one previous occasion (Garnham, P. C. C., personal communication). Parasitæmia rose to a peak of 75 parasites/1,000 red cells on the eighth day after the *MK* 116 inoculation. On the ninth, tenth, and eleventh days after this inoculation, the parasite counts were much lower, but gametocytes were prominent, at one time comprising 62 per cent of the total count of parasites.

On the eleventh day after inoculation, as the *P. cynomolgi* infection rapidly waned, a second species of *Plasmodium*, morphologically characteristic of *Plasmodium knowlesi*, suddenly appeared. It became apparent later that this second parasite resulted from the *MK* 116 sub-inoculation. The parasites of this species appeared in rapidly increasing numbers during the next three days. Two complete 24-hr. asexual cycles were observed, with maximum maturation of schizonts at about midday. On the evening of the fourteenth day after inoculation, the parasite count was 160/1,000 red cells. The monkey was severely anæmic, pale, and listless. At this time virtually all parasites in the blood-stream appeared to be *P. knowlesi*. 1 ml. of blood was inoculated into a clean *M. irus* (*MK* 117, previously treated with chloroquine and primaquine) to preserve the strain. This transfer was successful. At the same time 50 mgm. of sulphadiazine was administered intramuscularly in an attempt to save the life of the rhesus. This was unsuccessful; the monkey died from the overwhelming *P. knowlesi* infection during the night.

At the same time that these investigations were carried out, *P. cynomolgi* was isolated from *MK* 119 blood at the Memphis laboratory (confirming the suspicion that this species in rhesus *R* 614 had been derived from the first sub-inoculation), and *P. knowlesi* and *P. inui* were isolated from *MK* 116 blood. *P. knowlesi* became patent in Memphis rhesus *X* 509 five days after injection of blood from *MK* 116. For about a week small numbers of parasites were present. A very heavy *P. knowlesi* infection then developed which was successfully treated with chloroquine (50 mgm. a day intramuscularly for three days). Prior to treatment a second rhesus (*X* 536) was inoculated from *X* 509 and *P. knowlesi* infection developed rapidly. Treatment was carried out with sulphamerazine (250 mgm. a day by stomach tube for five days). The *P. knowlesi* parasites disappeared and typical *Plasmodium inui* became evident. Quartan periodicity was demonstrated by segmenter counts of daily blood films.

While *P. inui* was not detected in California rhesus *R* 614 in the face of the vigorous *P. knowlesi* infection, three of the nine other *M. irus* found infected appear to have plasmodia morphologically resembling *P. inui*. *P. inui* has been isolated from Philippine macaques on at least one previous occasion[1].

Plasmodium knowlesi has been recorded previously only from Malaya. The present report extends the known range of the parasite considerably. It is of interest, however, that a variant of *P. knowlesi* was discovered in Formosan macaques (*M. cyclopsis*) by Yokogawa *et al.* in 1941[2]. This variant, *Plasmodium knowlesi* var. *arimai*, was reported to have a 24-hr. asexual cycle, but to be only slightly or moderately pathogenic for rhesus monkeys, and non-infective for man. The Philippine strain of *P. knowlesi*, although not studied in man as yet, is certainly highly virulent in the rhesus and thus appears to be more closely related to the Malayan strain than to the Formosan variant.

Further work on these plasmodia is in progress at both laboratories; for convenience they have been termed the Cebu strains of *P. knowlesi*, *P. inui*, and *P. cynomolgi* respectively.

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Excretory Ducts in the Genus *Notopterus* (Lacepede)

THE reduction in size of the cœlom in the genus *Notopterus* has resulted in certain interesting modifications of the alimentary canal, swim-bladder, kidneys and gonads. An anomalous feature has also been observed in the organization of the kidney ducts and urinary bladder.

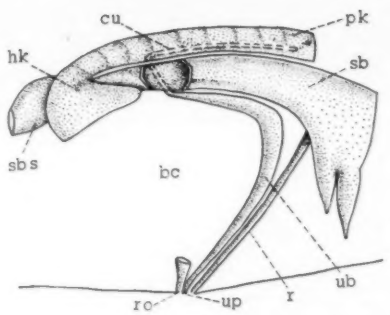

Fig. 1. Lateral view of the excretory organs and swim-bladder in *Notopterus*. *bc*, body cavity; *cu*, common ureter; *hk*, head kidney; *pk*, posterior kidney; *r*, first radial bone; *ro*, rectal opening; *sb*, swim-bladder; *sbs*, sub-spherical sac of swim-bladder; *ub*, urinary bladder; *up*, urinogenital papilla

The typical teleostean excretory organ consists of a pair of kidneys lying retroperitoneally beneath the vertebral column. Each kidney is differentiated into an anterior non-excretory part, the head kidney and a posterior functional part, the mesonephros. The ureter of each kidney proceeds backwards along the dorsal surface of the cœlom to open independently into the urinary bladder. In *Notopterus*, however, the ureters lie deep within the kidney. The two ureters join posteriorly to make a U-shaped bend whence a common, relatively thick-walled ureter runs forward superficially over the entire length of the left mesonephros. The common ureter then enters the swim-bladder, which lies immediately below, courses along its inner partition wall and pierces its tunica and fibrous peritoneal wall to open into a long, curved, tubular urinary bladder which extends as far as the middle of the body cavity.

The fusion of the ureters into a long common duct, its recurrent course along the kidney cephalad and its passage through the swim-bladder before it opens into the urinary bladder in both *Notopterus notopterus* and *Notopterus chitala*, the two available species in India, have not been reported earlier. The arrangement of the ureters and urinary bladder differs fundamentally from that of the typical teleostean excretory ducts.

Bridge[1], who worked on the swim-bladder of *Notopterus borneensis*, did not mention this feature. Also, Srivastava[2] and Dehadrai[3,4], who worked on the swim-bladder of *N. chitala*, failed to notice its association with the excretory system. It is inferred that an intense compression of the body and reduction of the cœlomic cavity have necessitated this change in the organization of kidney ducts and urinary bladder in the genus.

My sincere thanks are due to Dr. M. Chandy for her guidance and to Prof. B. R. Seshachar for helpful suggestions. I am also grateful to the Colombo Plan Authorities for the award of a Technical Co-operation Scheme Fellowship.

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Stimulants of Cell Division in Developing Fruits

STIMULANTS of cell division have been detected in a number of plant tissues, but little progress has been made in identifying the active substances involved[1]. Work on tobacco pith tissue culture[2] has recently shown that apple fruitlets collected while undergoing cell division contain particularly active stimulants of cell division. We have confirmed this, obtained further information about these stimulants and found that fruitlets of other species are also rich sources of factors stimulating cell division. Some properties of the factors in plum fruitlets have been determined.

The method of detection of these stimulants used in our experiments depended on observing the growth by cell division of explants from secondary phloem of carrot roots in the presence and absence of fruit extracts. Liquid culture methods[3] were employed. Ferric sodium ethylenediaminetetraacetate[4,5] was used as source of iron. All media contained 2 p.p.m. of indole-3-acetic acid, were adjusted to *p*H 6·4 and sterilized by autoclaving. Apple fruitlet extracts were active only in the presence of indole-3-acetic acid.

Sturmer Pippin apple fruitlets showed evidence of an active factor promoting cell division at the time of probable cessation of division (30 days from fertilization[6]) and again 24 days later. Activity was also detected in Granny Smith fruit 58 days from fertilization. No such activity, however, was detected in Sturmer Pippin fruit 110 days from fertilization or in mature fruit. The continued presence of stimulants of cell division in apple fruitlets after the cessation of intense cell division suggests that division is not regulated solely by the presence or absence of such stimulants. We have some evidence that extracts of more mature fruit inhibit the stimulative activity of the fruitlet extracts.

Stimulants of cell division were present in unfertilized fruitlets about to drop and in unfertilized fruitlets artificially held on the tree (by spraying with 2-benzthiazolyloxyacetic acid). Thus, while the development of an apple fruit is normally dependent on successful fertilization of a flower, the presence of stimulants of cell division in the developing receptacle is not entirely dependent on fertilization. Yet excision of the developing seeds, which are known to yield active extracts, renders the growth in sterile culture of fruitlet explants more dependent on the supply of a stimulant of cell division. Hence the developing fruit may depend in part on the developing seeds for a supply of such stimulants. The presence of stimulants has also been detected in other parts of the developing apple cluster (cluster base, fruitlet stalks and petals) and at times in leaves.

The presence of stimulants of cell division in fruitlets of other species collected within a period of 30 days from fertilization has been detected by the same method. In cold 70 per cent ethanol extracts of plum (diameter 7–11 mm.), peach (5–8 mm.) and pear (6–10 mm.) the activities equalled or exceeded that in similar apple fruitlet extracts and were about 40–50 per cent that of coconut milk. Less marked activity was detected in quince and pumpkin fruitlet extracts. No activity was detected in extracts of immature avocado or tomato fruit.

Some properties of the active substances in plum fruitlets have been determined. When the 70 per cent ethanol extracts were evaporated *in vacuo* at

Table 1. Weight and Cell Number of Carrot Tissue after Growth on Media containing Plum Fractions

Medium*	Final weight of explants† (mgm.)	Final cell number per explant† × 10^{-3}
Basal (*A*)	8·4	57
A + coconut milk (10 per cent by volume)	101·5	483
A + complete water-soluble fraction	13·7	252
A + fraction *AP*	55·6	461
A + fraction *EP*	4·6	43
A + *AP* + *EP*	17·6	279

* All plum fractions assayed at concentrations equivalent to 1 gm. fresh tissue per 100 ml. of medium.
† After growth for 21 days; each value is the mean for 20 explants.

40° C. and the residue extracted with water, all the activity was located in the water-soluble fraction. The activity of this fraction could not be accounted for by its content of amino-acids or chlorogenic acid, substances reported[1,7] to stimulate slightly the growth of carrot tissue. Attempts to concentrate the activity of this fraction by adsorption on, and elution from, charcoal were unsuccessful, even when the strongly adsorbing sites were blocked with palmitic acid. Extraction of an aqueous solution of the water-soluble fraction three times with a double volume of ethyl acetate at *p*H 3·2 yielded an aqueous phase (fraction *AP*) with enhanced activity and organic phases (fraction *EP*) containing factors which retarded both cell division and cell enlargement. The influence of these fractions on the growth of carrot explants is indicated in Table 1.

Fraction *EP* was rich in polyphenolic compounds, two of which have been tentatively identified by paper chromatography as catechin and *epi*-catechin. These two substances have been found to retard the growth of carrot tissue. The phenolic components of fraction *EP* may account for the inhibitory action of this fraction. Ethyl acetate extraction would appear to be a simple, effective method for removing some growth inhibitors from plant extracts containing natural stimulants of cell division. (*Note added in proof.* Sumicki and Kawarda have also used ethyl acetate for this purpose, *Plant Growth Regulation*, 483; Iowa State Univ. Press, 1961).

Extraction by butanol and ion-exchange investigations have both provided evidence for the presence in the plum extract of at least two synergistic factors promoting division. Fraction *AP* was further fractionated by running first through 'Zeo-Karb 225' (H) and then through 'Amberlite *IR*-4*B*' or 'De-Acidite *G*' (free base). Activity was confined to the fraction obtained by elution of the 'Zeo-Karb' with 1·5 *N* ammonia after first removing adsorbed material with 70 per cent alcohol, and to the fraction not adsorbed on either resin. Activity of each was slight, but in combination they yielded 90 per cent of the original activity.

The factors in plum fraction *AP* differ from kinetin both chemically and biologically. Extraction with diethyl ether at *p*H 7 yielded inactive ether extracts, all the activity being accounted for by the extracted aqueous phase. Kinetin would be extracted by ether[8]. Fraction *AP* will not replace kinetin in satisfying the non-photosynthetic red-light requirement of *Spirodela oligorrhiza* (analogous to that of *Lemna minor*[9]) and in this respect resembles coconut milk. The factors in plum extract also resemble those in coconut milk in that they can be divided by ion exchangers. However, the 'neutral' fraction from coconut milk is itself completely inactive[1], whereas that from plum extracts promotes division slightly. The activity (increase in cell number per unit dry weight) of plum fraction *AP* is ten times that of coconut milk.

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Flower Initiation in Kent Wild White Clover (*Trifolium repens* L.), under Controlled Environmental Conditions

Field experiments on seed production in white clover carried out here during 1958–60 have emphasized the importance of time of flowering in relation to grazing management[1].

Under natural field conditions the first signs of flower initiation can usually be seen during the third week in April, although in spaced plants flower initials are laid down earlier. During this period longitudinal sections of the apex of the stolon show the developing inflorescence as a flattened mass of meristematic tissue in the axil of the subtending leaf close to the growing point of the stolon. This meristematic region rapidly develops into a cone-like structure, differentiating individual florets from the base upwards[2,3].

Little critical work has been done on the environmental factors controlling flower initiation in white clover. Laude[4] investigated the effect of photoperiod on flowering in Ladino white clover, and compared plants overwintered outdoors with plants overwintered in heated glasshouses. Cains[5] has observed the effects of seed vernalization on flowering in white clover. Britten[6] has shown that warm-day, cold-

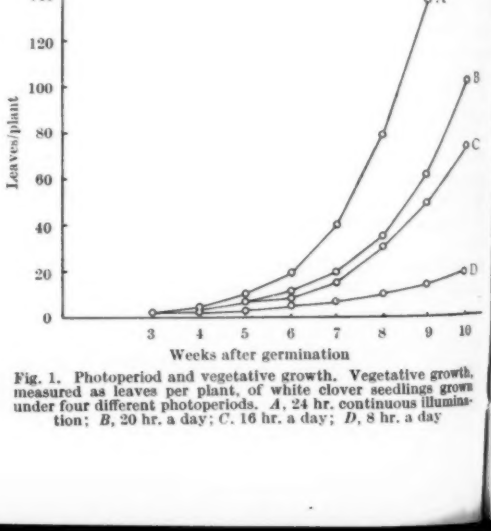

Fig. 1. Photoperiod and vegetative growth. Vegetative growth, measured as leaves per plant, of white clover seedlings grown under four different photoperiods. *A*, 24 hr. continuous illumination; *B*, 20 hr. a day; *C*, 16 hr. a day; *D*, 8 hr. a day

Table 1. PHOTOPERIOD AND FLOWERING

Photoperiod	Time to first-flowering (days)	No. of inflorescences per plant
24 hr.	67	8·0
20 hr.	102	5·0
16 hr.	115	2·0
8 hr.	—	0·0

night temperatures can promote flowering in certain 'non-flowering' clones of white clover.

In the present investigations the effect of photoperiod and vernalization on the growth and development of wild white clover plants has been investigated, using controlled growth-room conditions (unpublished results).

Length of day has been found to be the chief environmental factor controlling flowering in white clover. When plants were grown from seed under photoperiods of 8 hr., 16 hr., 20 hr. and continuous illumination at 500 foot-candles and 65° F., flower initiation was observed only in plants in photoperiods of 16 hr. or more. In day-lengths greater than 16 hr. there was a quantitative response in both vegetative growth (Fig. 1) and reproductive development (Table 1) reaching a maximum under continuous illumination. This long-day flowering response in white clover confirms the findings of Laude[4], although he obtained relatively little difference in response to photoperiods of 15½ hr. or greater.

Cold temperatures during seed germination were not found to be essential for subsequent flowering, since some plants grown from non-vernalized seed completed their full cycle of development under long-day photoperiods. However, seed vernalization at 38° F. for 15 days resulted in earlier flowering and increased the number of inflorescences per plant, when grown on under natural long-day photoperiods in the early summer. Vernalization at 38° F. for longer, or shorter, periods than 15 days appeared to depress flowering ability.

Preliminary results for the interaction of seed vernalization and photoperiodism suggest that vernalization becomes more important in promoting flowering in the shorter photoperiods. In this respect, white clover appears to behave similarly to subterranean clover, as reported by Evans[7].

Once flowering has taken place, vegetative plant material has been induced to flower again by limited periods of cold temperature. Vegetative stolon cuttings were given successively longer periods of cold temperature in an unlit refrigerator at 38° F. When grown afterwards in the growth-room at 65° F., flowering occurred earliest under long-day photoperiods from plants which had received 1–2 weeks of cold temperature.

Under field conditions there would appear to be a positive flowering response as a result of the cold temperature received during the autumn and early winter. Plants that had received successively longer periods of natural low temperature during the autumn tended to flower earlier when brought into the growth-room and grown under long-day photoperiods at 65° F. This effect of cold temperature appeared to be satisfied by the beginning of January, since plants brought in thereafter showed little further increase in flowering response.

In the field it has been observed that some plants initiate flowers early in the spring and continue producing flowers for several weeks, whereas other plants may start flower initiation later in the spring and continue flowering for only a few days. This wide range of response is probably genetical and offers considerable scope for plant selection for time of flowering.

These findings have shown that Kent wild white clover is a long-day plant and that flowering can be stimulated by limited periods of cold temperature.

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MICROBIOLOGY

Metabolic and Genetic Investigations of Nutritionally Exacting Strains of *Staphylococcus pyogenes*

COAGULASE-POSITIVE strains of *Staphylococcus pyogenes*, taken at random from material phage-typed at Hammersmith Hospital and at the Central Public Health Laboratory, Colindale, were tested for their nutritional character. All but 50 of the 926 strains grew well on a defined medium (based on that of Fildes *et al.*[1]) containing valine, leucine, glycine, proline, aspartic acid, methionine, phenylalanine, arginine, histidine, cysteine, thiamine and nicotinic acid in a glucose-citrate-salts-agar base. Of the 50 exacting strains, all but 4 grew on the medium further supplemented as follows: tryptophan, 12 strains; threonine, 6 strains; alanine, 4 strains; tyrosine, 3 strains; lysine, 2 strains; tryptophan and tyrosine, 9 strains; tryptophan and *iso*leucine, 2 strains; tryptophan and biotin, 2 strains; threonine and glutamic acid, 1 strain; biotin, 2 strains; riboflavine, 1 strain; hypoxanthine, 2 strains.

Non-exacting variants were obtained from many of the exacting strains when large inocula were plated on the defined medium. The strain requiring threonine and glutamic acid gave variants able to dispense with threonine, and others able to dispense with glutamic acid, but none able to dispense with both amino-acids. This suggests that the two requirements result from two genetic lesions, each capable of repair by mutation. Some strains yielded only small-colony-type non-exacting variants, like those resulting from suppressor mutations in some nutritionally exacting mutants of *Salmonella typhimurium*.

Of the strains responding to tryptophan, some responded to anthranilic acid, indole or tryptophan, some to indole or tryptophan, some only to tryptophan; these categories correspond to classes of auxotrophic mutants in *Salmonella typhimurium* with blocks at different points in the biosynthetic path to tryptophan. None of the strains requiring both tryptophan and tyrosine responded to shikimic acid, the common precursor of the three aromatic amino-acids (of which phenylalanine was present in the standard medium); furthermore some of these strains utilized indole, and some indole or anthranilic acid, in place of tryptophan. It is therefore unlikely that they owe their double requirements to a single block in the synthesis of the aromatic amino-acids.

Of the 7 strains responding to threonine, or threonine plus glutamic acid, 2 only responded to homoserine, a precursor of threonine and methionine in *Escherichia coli*[3]. These 2 strains, but not the other 5, failed to grow on threonine-supplemented defined medium from which methionine had been omitted. These 2 strains, needing either threonine and methionine or homoserine, are, we infer, unable to convert aspartic acid to homoserine; and the other 5 threonine-requiring strains unable to convert homoserine to threonine.

We attempted to obtain non-exacting forms from the exacting strains by phage transduction, using typing phage 53, and other typing phages serologically related to it, obtained by growth on their respective propagating strains, all of which grew on our defined medium; Morse[3] showed that phage 53 obtained by induction of a lysogenic strain transduced fermentation and drug-resistance characters. Transduction of the non-exacting character was observed in all the strains responding to threonine, and in some with requirements for tryptophan, tyrosine, alanine, lysine or hypoxanthine. In the threonine-exacting strains with a phage-bacterium input ratio of 5 : 1 about 10^{-7} transductant colony was obtained per phage particle; the rate was lower in the other strains. No minute colonies, such as result from abortive transduction, were seen.

Phage 53 was grown on the two strains responding to threonine or homoserine, and on spontaneous non-exacting variants of them. When either of the exacting strains was treated with phage grown on the other, the yield of non-exacting colonies was only about one-fifth of that evoked by phage grown, instead, on the non-exacting variant. We infer that these 2 strains have non-identical genetic lesions, at sites very closely linked so that their probability of co-transduction is about 0·8. As phage 53 could not be grown on the 5 strains responding only to threonine these strains could only be used as receptors. When any of the 5 was treated with phage grown on either of the 2 strains responding to threonine or homoserine about half the colonies obtained by plating on defined medium supplemented with homoserine had the requirement for homoserine (or threonine) of the donor strain. We infer that the sites of the genetic lesions in the 5 threonine-requiring strains are closely linked to the sites in the homoserine-responding strains, so that the probability of co-transduction is about 0·5.

Thus examination of the minority of strains of *Staph. pyogenes* which are nutritionally more exacting than the majority has provided evidence that the biosynthetic paths to tryptophan and to threonine are similar to those in *Salm. typhimurium* and *Esch. coli*; and that typing phage 53 and some serologically related phages can transduce various nutritional characters. The observed linkage between loci for successive steps in the conversion of aspartic acid to threonine has been found also in *Salm. typhimurium*[4].

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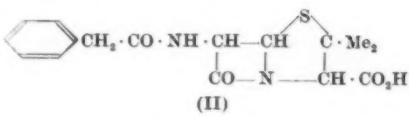
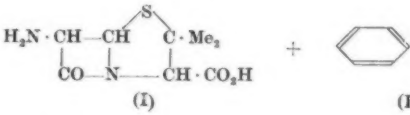
A Method for isolating Bacteria capable of producing 6-Aminopenicillanic Acid from Benzylpenicillin

Batchelor *et al.* discussed the isolation of 6-aminopenicillanic acid (I) from a submerged culture of *Penicillium chrysogenum*[1]. Recently, it was shown that (I) could be prepared by the microbial hydrolysis of benzylpenicillin (II)[2–4].



(II)



(I) (III)

While engaged in the study of resolving amino-acids by metabolism or hydrolysis of soil bacteria on acyl derivatives of DL-amino-acids[5–7], we have found that more than eight strains (*KT* 514, *KT* 513, *KT* 515, etc.) of soil bacteria capable of producing (I) from (II) by metabolism or enzymic hydrolysis could be isolated from four different field areas by using a synthetic medium containing (II) as the sole source of carbon.

The constituents of the culture medium used in the experiments are as follows: potassium benzylpenicillin, 0·93 gm. (0·0025 mole); NH_4Cl, 0·1 gm.; K_2HPO_4, 0·1 gm.; $MgSO_4 7H_2O$, 0·05 gm.; 1 per cent $CaCl_2 6H_2O$, 2 drops; 1 per cent $FeCl_3 6H_2O$, 1 drop; distilled water, 100 c.c.; *p*H 7·0–7·2. All strains (*KT* 514, *KT* 513, *KT* 515, etc.) could be cultivated successively in the synthetic medium containing phenylacetic acid (III) as well as (II) as the sole source of carbon. They seemed to be rod-shaped in the phenylacetic acid medium, but they had a tendency to form filaments in the benzylpenicillin medium.

When 18·6 mgm. of potassium benzylpenicillin was incubated with 20 mgm. of bacterial acetone powder in 2 c.c. of 0·1 *M* potassium phosphate buffer at *p*H 7·8 and 37° C. for 4 hr. in the presence of toluene, the reaction mixture was found to contain 1·12–0·17 mgm. (that is, 10·4–1·6 per cent of the theoretical quantity) of (I), and (III) (Table 1). (I) was determined by treating a centrifuged sample with phenylacetyl chloride and bio-assaying the (II) formed by a plate diffusion assay. (III) was tested by the method of Pan *et al.*[8].

When ammonium chloride was removed from the benzylpenicillin medium, *KT* 514, *KT* 513, *KT* 515, and *KT* 512 could not or only just be cultivated, and then they scarcely utilized (I) as the sole source of

Table 1. Production of 6-Aminopenicillanic Acid from Benzylpenicillin by Hydrolysis of Bacterial Acetone Powder

Strains of soil bacteria	6-Amino-penicillanic acid (per cent)	Phenyl-acetic acid	Strains of soil bacteria	6-Amino-penicillanic acid (per cent)	Phenyl-acetic acid
KT 502	2·6	+	*KT* 513	2·3	+
KT 504	6·5	+	*KT* 514	1·6	+
KT 510	5·2	+	*KT* 515	10·0	+
KT 511	10·4	+			
KT 512	7·8	+	*KT* 517	2·6	+

Table 2. PRODUCTION OF 6-AMINOPENICILLANIC ACID FROM BENZYLPENICILLIN BY METABOLISM OF BACTERIA

Strains of soil bacteria	Fermentation time (day)	6-Aminopenicillanic acid (per cent)
KT 514	3	6·0
KT 513	3	5·5
KT 515	3	3·7
KT 512	3	1·5

carbon in the presence of ammonium chloride. Consequently, it is to be understood that *KT* 514, *KT* 513, *KT* 515, and *KT* 512 may produce (I) from (II) by utilizing (III), which was formed from (II) by the action of their 'penicillin acylase', as the sole source of carbon and ammonia as the sole source of nitrogen. A loop of the culture fluid was inoculated into 5 c.c. of the culture medium containing potassium benzylpenicillin and incubated at 25° C. for three days. The fermentation liquor contained 1·62–0·4 mgm. (that is, 6·0–1·5 per cent of the theoretical amount) of (I) (Table 2). The production of (I) was assayed by the microbiological determination mentioned here.

Thus, a new method to isolate bacteria capable of producing (I) from (II) was established.

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Leptospirosis of White Laboratory Rats

LEPTOSPIRAL infection of white laboratory rats was first reported by Uhlenhuth and Zimmermann[1]. Later it was observed in some stocks in Germany, Holland and Denmark[2]. The infection seemed to be present inapparently in white rats, although some observers emphasized the high incidence of albuminuria in these populations. The examination of single isolated strains and the results of serological screening tests suggested *Leptospira icterohaemorrhagiae* as the causative agent. It was generally assumed that the infection originated from wild rats in the animal house. Human cases were regularly detected in these institutes showing mainly the picture of Weil's disease with a high fatality-rate.

Our investigations in Hungary in 1959 and 1960 showed that leptospiral infection often occurs in large stocks of Wistar rats. The animal sera gave positive sero-reactions of high titre with the sero-types of the *icterohaemorrhagiae* sero-group; with other sero-types only occasional cross-reactions were present. The frequency of sero-positivity varied in the different populations, but in some stocks it ranged to 100 per cent in old animals. The positive sero-reactions indicated that the rats were carriers and *Leptospirae* could be easily cultured from the kidney of these animals. Study of the isolated strains showed that they represent one sero-type, which is closely related to the sero-types of the *icterohaemorrhagiae* sero-group, but they could be differentiated in cross-absorption tests. Therefore the name *Leptospira budapest* was given this sero-type, indicating the place of the first description.

Epidemiological investigations suggested the endemic spread of the infection in different white rat populations. In agreement with our former observations, typical *icterohaemorrhagiae* strains could be isolated from wild rats in Hungary and *Leptospira budapest* was encountered hitherto only in white laboratory rats.

The widespread incidence of leptospiral infection in white rats directs attention to the paramount importance of this problem in institutions where white rats are regularly used for experimental and routine work. Epidemiological surveys and serological screening tests generally revealed human cases in such places, occurring mainly among personnel of the animal house. The presence of the infection in the stocks may affect the experimental results in diets, kidney and liver function tests, determination of the basal metabolism and in histological examinations.

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GENETICS

Stabilizing Selection for Heading Time in a Bulk-Hybrid Population of Barley

SEVERAL workers[1–3] have recently pointed out the importance of the stabilizing type of natural selection, in which the intermediate phenotype has an adaptive advantage over the deviants from such a centrally located norm, thus resulting in a rather stable population mean and a continually diminishing population variance. Robertson[4] has analysed the theoretical implications of two different models of stabilizing selection: the intermediate optimum model of Wright[5], where the more fit individuals are phenotypically intermediate, and the homeostatic model of Lerner[6], in which the favoured individuals are more fit since they are heterozygotes. The latter model involves true overdominance at one or more loci governing fitness or its various components. Hence, it would be of interest to investigate an actual example of stabilizing form of selection for its fit to one or the other model. The present communication summarizes evidence for stabilizing selection for heading time (recorded as the number of days before the first spike comes out of the flag leaf) in a bulk-hybrid population of barley that was derived from a composite cross and propagated over 18 generations under natural self-fertilization without conscious selection, and it further provides some information on the discriminating choice between the two models.

The experimental material consisted of the random samples of single plants in the bulked F_3, F_5, F_6, F_{13}, F_{15}, and F_{18} generations along with all the thirty

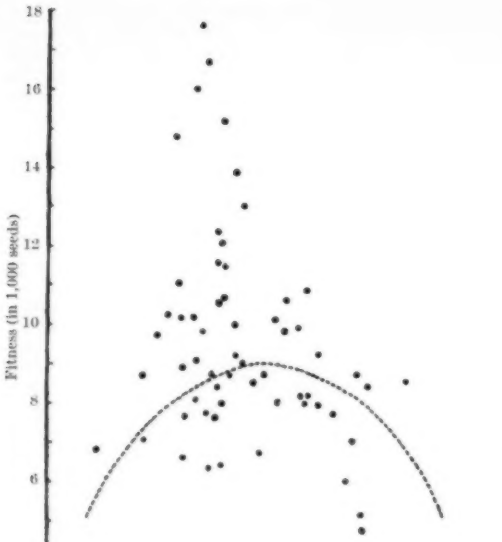

Fig. 1

parents of the composite cross, all planted together in a five-times replicated randomized block design. Table 1 gives the estimates of overall generation means and variances. A relatively small change in the mean towards earliness together with a consistent and striking diminution of the variance clearly suggest a strong pressure of stabilizing selection for intermediate metric values of heading date, since in absence of selection, theoretically, continued inbreeding is expected to bring about an increase in total variance through having subdivided a population into many familial lines. Wright[7] showed that with all genetic variance additive, the among-family-means and within-families variances are simple, linear functions of the inbreeding coefficient, F, such that σ_m^2 (among-families variance) $\propto 2F$, and σ_w^2 (within-families variance) $\propto (1-F)$. The relative proportions of σ_m^2 and σ_w^2, as estimated from progenies of 63 randomly chosen individuals in each of the F_3, F_6, F_{13}, and F_{18} generations, are given in Table 2. For sake of comparison, their estimates from the thirty parental cultures are also included. The values of σ_m^2 indicate a steady decrease in the amount of genetic heterogeneity due to the extinction of certain families under inbreeding and selection. The within-families variance, σ_w^2, is smallest in the parental (P) generation, and although it decreased slowly with inbreeding generations, yet F_{19} gave a higher value of σ_w^2 than P, an interesting observation that might be accounted for by a certain amount of heterozygosity being retained by natural selection even after as many as 18 generations of 98–99 per cent selfing occurring in this material[8]. The frequency distribution for the individual progenies shows only continuous variation which might imply that perhaps none of the segregating and overdominant loci could be singled out as being the major one. It is significant to note, however, that earlier, Jain and Allard[8] reported evidence for a distinct heterozygote advantage at four different marker loci segregating in the present material. The homeostatic model of stabilizing selection deals with precisely such truly overdominant loci.

Table 1. Estimates of Overall Generation Means and Variances

Statistic	Generation F_3	F_6	F_{13}	F_{18}	F_{15}	F_{19}
Mean (days)	18·86	16·94	17·42	16·27	16·47	16·42
Variance	31·76	24·06	25·14	21·75	21·05	18·00

Table 2. Estimates of Among-Families (σ_m^2) and Within-Families (σ_w^2) Variances

Statistic	Generation F_6	F_{13}	F_{18}	P
σ_m^2	745·61	462·58	528·13	398·36	1,280·97
σ_w^2	10·70	9·80	8·91	7·10	5·28

A further consequence of the homeostatic model can be verified from the present experiment. As pointed out by Robertson[4], unlike its alternative model, the homeostatic model implies that homozygosis would never be complete and that the fitness (measured by the number of seeds produced) and mean heading time of a family would not be correlated. The fitness – heading time graph for F_{19} progenies (Fig. 1) actually shows a very wide scatter of points with reference to the parabolic curve expected under the intermediate optimum model. The absence of any significant correlation here may therefore be taken as further evidence for heterozygote advantage as the basis of stabilizing selection. A detailed report of this experiment is hoped to be published elsewhere.

I wish to thank Mr. C. A. Suneson of the United States Department of Agriculture for providing the seed material, and Prof. R. W. Allard of the University of California, Davis, for his helpful supervision and criticism. This work was carried out at the University of California, Davis, California.

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Pulmonary Tumours and Group VII in the House-Mouse

Certain aspects of the experiments reported in Dr. J. A. H. Tatchell's recent communication[1] are likely to result in misunderstanding of the genetic nature of the inheritance of susceptibility to pulmonary tumours in mice. Heston[2] has established that the inheritance of susceptibility to pulmonary tumours in mice is multifactorial in nature and therefore the further investigation of this problem requires an approach designed to take this into account. The experiment reported by Dr. Tatchell does not appear to do so.

I agree that Heston's[3–5] evidence proves the presence of a factor in linkage group VII, but it has not been shown that this is the only factor influencing susceptibility to pulmonary tumours in this linkage group; nor has the possible existence of background effects resulting from segregating loci present in other linkage groups been taken into account. The validity

of estimating the recombination frequency depends on the supposition that the classification by the presence or absence of tumours corresponds with the segregation of a single Mendelian gene, and Dr. Tatchell has not given any supporting evidence for this. Even if the classification resulted in a 1:1 segregation in backcross matings, this in itself would not be sufficient evidence because, as Wright[6] showed in a well-known paper on polydactyly in guinea pigs, a polygenic character with a threshold, and all-or-none expression, can "simulate one-factor Mendelian heredity to a remarkable extent" in F_1, F_2 and back-cross segregations. But the single-factor interpretation "breaks down completely in the tests of the supposed segregants". In addition, because of the segregation of genes at other loci, some mice with the linkage group VII gene may have no tumour, and some mice without the gene may have tumours. Dr. Tatchell states that there was certainly some degree of misclassification but does not indicate how much. Without a knowledge of the accuracy of the classification it is impossible to estimate the true recombination frequency: the results obtained are equally compatible with either loose linkage and accurate classification or close linkage and inaccurate classification.

A further complication is that the treatment used resulted in a 50 per cent loss of experimental animals, which appears to be excessively high; no information is given as to whether or not this loss is randomly distributed between the mutant groups. If the loss were not randomly distributed, so high a mortality might have seriously affected segregation ratios and the apparent recombination frequencies.

In my opinion consideration of these criticisms leads to the conclusion that the approach that Dr. Tatchell has used in this experiment is not valid for this character.

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THE points raised by Dr. Bloom were either fully covered in my communication or else were unsuitable for inclusion in a preliminary account.

I stated that Heston's work *suggests* the presence of one major factor in linkage group VII without excluding minor factors; I did not claim that there is definitely only one factor involved, and I pointed out that his work does not exclude some interaction.

I agree that a polygenic character can "simulate one-factor Mendelian heredity to a remarkable extent", but Fisher's[1,2] later work on polydactyly in mice has clearly shown not only that a major gene can be isolated from its modifiers by refined genetic techniques but also that the demonstration of linkage with another factor is unassailable confirmation of its existence. He has also shown the necessity for having both phases before the evidence for linkage itself is conclusive. This is precisely why I have stated, on one hand, that the lack of linkage between the possible factor (*Tm*) and sex, where both phases are available, is proved, while on the other hand, the putative linkage between *Tm* and group VII, where the coupling phase only is available, is still uncertain. The further experiments in this series are an attempt to give the second phase (repulsion), as stated in my communication. This attempt is itself a test of "the single-factor interpretation" because the repulsion heterozygotes will be "supposed segregants", and thus, if they in turn give single-factor ratios, this will be evidence, additional to linkage, of the presence of the major factor.

The degree of modification of *Tm*, and thus the degree of misclassification, should also be more accurately measured by this further experiment. However, misclassification on the scale proposed by Dr. Bloom would already have been revealed by the more critical analysis which, as I have stated, I will give in my more detailed account. As this analysis agrees well with the simpler treatment, I felt that the latter was more suitable for publication in a preliminary communication, particularly as this was the only one used by previous workers. With minimal misclassification and sufficient numbers, it may now be claimed not only that Heston's "association" with group VII is strongly confirmed but also that, with the choice of factors having reduced known weight interaction, the case for linkage is strong enough to be worth pursuing.

I regret that my footnote to Table 2 did not indicate that the apparently high mortality-rate was due to my having to kill large numbers of my stock because of circumstances beyond my control and unconnected with the experiment. It includes also, of course, the low random litter mortality present in any breeding stock. The mortality-rate after injection was, in fact, extremely low.

In my opinion the application of genetic techniques and mathematical analyses used successfully in other apparently polygenic situations is essential to the discovery of a single major factor in cancer work and that no advance in this direction can be made without it.

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Distribution of X-ray- and Nitrous Acid-induced Mutations in the Genetic Fine Structure of the ad_7 Locus of *Schizosaccharomyces pombe*

GENETIC research of the past ten years has shown that the classical gene can be subdivided by mutation, recombination and function. The region of a chromosome which determines the production of one enzyme can be inactivated by mutation at many independent sites which are separable by recombination. One well-known example is the *r*II region of phage *T*4 (ref. 1. For other genes analysed in micro-organisms see Pontecorvo[2] and Demerec and Hartman[3]).

In fungi, one of the most extensive intragenic recombination analyses has been performed by Leupold in the ad_7 locus of the yeast *Schizosaccharomyces pombe*[4a–c]. Leupold has obtained 153 ad_7 mutants by ultra-violet irradiation. These mutants need adenine for growth and accumulate a red pigment. Most of the ultra-violet mutants cannot grow without adenine at any temperature tested, but six

mutants are sensitive to temperature (no growth at 25° C., growth at 35° C.). Using intragenic recombination analyses, Leupold has constructed a linear map of the ad_7 locus (Fig. 1*a*). The ultra-violet mutants are not randomly distributed on the map. There are a few 'hot spots', and the mutation sites appear to be concentrated in several parts of the map. Each ultra-violet mutant seems to be a 'point mutation'.

It was of interest to test the action of other mutagenic agents on the ad_7 locus. First, I chose X-rays. They are known to produce chromosomal aberrations, for example, deletions, and I wanted to see whether gross changes of corresponding types are produced on the intragenic level as well. Secondly, I chose nitrous acid. Nitrous acid causes inactivation and mutations in tobacco mosaic virus[5], bacteriophages[1,6], and bacteria[7]. *In vitro*, it deaminates the purine and pyrimidine bases of the ribonucleic acid[5] and deoxyribonucleic acid (DNA)[8]. Thus nitrous acid is a very interesting mutagen which is able to react directly with the genetic substance by a known mechanism. With respect to the ad_7 locus, one would expect to obtain mutations which are distributed evenly within the map, without the expression of 'hot spots'. In bacteriophage *T*4, however, Benzer[1] has found that the intragenic distribution of nitrous acid-induced rII mutants is non-random, suggesting that mutation-rates at individual sites may depend not only on the nature of the base pair involved but also on its position with respect to neighbouring base pairs. In cellular organisms, the situation is complicated further by the fact that the mutagenic action of nitrous acid may in part be an indirect one, affecting the DNA through changes in metabolism rather than by a direct deamination of its amino-bases.

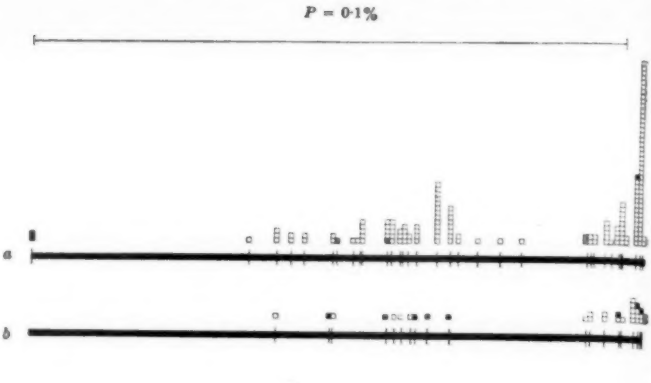

Fig. 1. Map of the ad_7 locus of *Schizosaccharomyces pombe* showing the distribution of the mutants induced by *a*, ultra-violet light (according to Leupold (ref. 4*c*); *b*, X-rays; *c*, nitrous acid. The map has a length of about 0·1 Morgan unit. ⊠, Temperature-sensitive; ■, mating type

In my X-ray experiments most of the mutants were produced with 180 keV. X-rays at a dose of 30,000 r. For the experiments with nitrous acid we used cells which were starved for 6 hr. in saline. The cells were incubated in acetate buffer *p*H 4·0 plus 0·03 *M* sodium nitrite at 30° C. for 3–5·25 min. This results in an inactivation of about 40–90 per cent. The treated cells were plated on yeast-extract agar. Following incubation at 30° C. the plates were scored for red colonies. I obtained 32 ad_7 mutants with X-rays and 51 with nitrous acid. The mutants were examined by the methods described by Leupold[4a]. The results of the intragenic recombination analyses are represented in Fig. 1*b* and 1*c*.

Each of the 32 X-ray mutants appears to occupy just one site only, no multi-site mutations having been obtained; they have to be considered as 'point mutations'. The distribution of the mutants in the map is similar to that of the ultra-violet mutants. Thus, no striking difference is observed in the distribution of ad_7 mutants induced with ultra-violet light or X-rays. Seven X-ray mutants have not only mutated in the ad_7 locus but also from mating type + to mating type −. It is not yet clear whether there is any causal connexion between the ad_7 mutations and the mutations concerning the mating type.

The ad_7 mutants obtained with nitrous acid are quite different from the ultra-violet and X-ray mutants. 29 out of the 51 mutants are sensitive to temperature. It is very striking that they show pronounced 'hot spots' different from those of the ultra-violet and X-ray mutants (Fig. 1*c*). It should be mentioned especially that no nitrous acid-induced mutations were found at the 'hot spots' on the right end of the ultra-violet map, and that a 'hot spot' of temperature-sensitive mutants is present in the left part of the map, at a site at which neither ultra-violet nor X-ray mutations has been observed.

The fact that nitrous acid-induced mutants show 'hot spots' may be interpreted as indicating that, under our experimental conditions, the nitrous acid has not attacked the DNA directly or only partially so. The mutagenic action of nitrous acid may to a certain degree be an indirect one, inactivating the ad_7 locus at specific sites partially different from those attacked by ultra-violet and X-rays.

The greater part of the work reported was performed during a research visit at the Institute of General Botany, University of Zurich, Switzerland, and has been supported by grants of the Deutsche Forschungsgemeinschaft, the Schweizerische Nationalfonds, and the Julius-Klaus-Stiftung, Zurich.

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FORTHCOMING EVENTS

Thursday, September 14—Friday, September 15

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at the Royal Society of Medicine, Wimpole Street, London, W.1), at 9.30 a.m. daily—Symposium on "Recent Advances in Processing Cereals".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

EXPERIMENTAL OFFICER (with a general degree with zoology, botany and chemistry) IN THE DEPARTMENT OF INSECTICIDES AND FUNGICIDES—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (September 15).

LECTURER (with high academic qualifications and also teaching or research experience) IN INORGANIC AND PHYSICAL CHEMISTRY—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18 (September 15).

LECTURER (with suitable academic and/or industrial experience) IN MATHEMATICS—The Secretary, Royal College of Science and Technology, Glasgow (September 15).

SCIENTIFIC OFFICERS/SENIOR SCIENTIFIC OFFICERS (with a first- or second-class honours degree in a science or engineering subject, and a wide and intelligent interest in scientific matters and an aptitude for administrative work) at the Headquarters Office of the D.S.I.R., Regent Street, London, for scientific administrative work—The Establishment Officer, Headquarters Office, D.S.I.R., 5–11 Regent Street, London, S.W.1, quoting HQ.4 (September 16).

ASSISTANT HYDROGRAPHER (marine zoologist or a geographer with suitable training and experience), for work in connexion with an investigation by a small team of the physical, chemical and biological conditions of inshore waters, with particular reference to the biology of sessile marine organisms—The Registrar, University College of North Wales, Bangor, North Wales (September 18).

DIRECTOR OF COMBINED STUDIES IN THE FACULTY OF SCIENCE, to supervise admissions to and the progress of students in the courses leading to the newly instituted degree in which two science subjects are studied to a higher level—The Registrar, The University, Leeds 2 (September 18).

HEAD (well qualified in botany or zoology and with appropriate teaching, research and industrial experience) OF THE DEPARTMENT OF BIOLOGY—The Principal, Brunel College of Technology, Woodlands Avenue, Acton, London, W.3 (September 18).

RESEARCH STUDENT IN PSYCHOLOGY—The Registrar, The University, Hull (September 18).

HISTOLOGICAL TECHNICIAN, Experimental Officer grade, to be in charge of a service to research workers involving a wide range of methods for research programmes—The Secretary, Institute of Animal Physiology, A.R.C., Babraham, Cambridge (September 23).

RESEARCH ASSISTANT IN THE DEPARTMENT OF BOTANY, Gatty Marine Laboratory, St. Andrews, to assist in cultural, cytological and taxonomic investigations of marine algæ—The Secretary of University Court, College Gate, St. Andrews (September 23).

ASSISTANT LECTURERS (2) IN ORGANIC, INORGANIC OR PHYSICAL CHEMISTRY—The Registrar, The Manchester College of Science and Technology, Manchester 1 (September 25).

LECTURER (with an honours degree in electrical engineering or physics and experience in some branch of electrical or electronic engineering) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—The Registrar, The University, Leeds 2 (September 25).

SENIOR LECTURER or LECTURER IN APPLIED MATHEMATICS at the University of Ghana—The Assistant Registrar (London), University of Ghana, 15 Gordon Square, London, W.C.1 (September 25).

DEMONSTRATOR IN AGRICULTURE—The Assistant Bursar, The University, Reading (September 29).

PRINCIPAL LECTURER IN POLYMER CHEMISTRY AND TECHNOLOGY in the Department of Chemistry, Metallurgy and Textiles, to teach in H.N.C., Grad. R.I.C. and Dip.Tech. standard courses—The Registrar, Lanchester College of Technology, Priory Street, Coventry (September 29).

JUNIOR LECTURER (preferably interested in cytology, radiation effects, or other aspects of biology with an application to medicine) IN THE DEPARTMENT OF ZOOLOGY—The Secretary, The Medical College of St. Bartholomew's Hospital, West Smithfield, London, E.C.1 (September 30).

LECTURER IN CYTOGENETICS IN THE DEPARTMENT OF AGRONOMY, Waite Agricultural Research Institute, University of Adelaide, Australia, to undertake cytogenetic studies as part of a cereal breeding and genetic research programme—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 30).

LECTURER IN THEORETICAL PHYSICS; a LECTURER IN PHYSICS; and ASSISTANT LECTURERS IN PHYSICS—The Registrar, The University, Manchester 13 (September 30).

PLANT PHYSIOLOGISTS (2) (honours graduates with research experience in plant physiology) at the Tobacco Research Institute, Division of Plant Industry, C.S.I.R.O., Mareeba, to conduct research into the physiology of the tobacco leaf within one of the following fields: (*a*) morphogenesis, particularly leaf development; (*b*) water relations; or (*c*) photosynthesis and translocation—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 815/33 (October 2).

LECTURER IN BIOCHEMISTRY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 13).

CHAIR OF CHEMISTRY in the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 15).

LECTURER IN MATHEMATICS at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 16).

LECTURER or SENIOR LECTURER IN MATHEMATICS at the University of Tasmania, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 16).

LECTURER IN CIVIL ENGINEERING at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 20).

LECTURER/SENIOR LECTURER IN APPLIED MATHEMATICS at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 20).

SENIOR LECTURER IN PHYSICS in the School of General Studies, Australian National University, Canberra—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 21).

FELLOW IN THE DEPARTMENT OF DEMOGRAPHY, Institute of Advanced Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 27).

ASSISTANT LECTURER (Grade B) IN MECHANICAL ENGINEERING—Clerk to the Governing Body, Borough Polytechnic, Borough Road, London, S.E.1.

CHEMIST (male, with an honours degree in chemistry or an equivalent qualification, and not less than two years postgraduate experience or training) IN THE DEPARTMENT OF AGRICULTURE, Tanganyika, for research work on coffee, including soil and plant analysis connected with nutritional problems and soil survey work—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting BCD.63/8/020/T.

FISHERY RESEARCH OFFICER (male, aged 22–35, with a good honours degree in natural science (zoology), some training in fish ecology, and preferably experience of handling nets and some acquaintance with hydrographic gear) in Nyasaland, for research into the biology and ecology of Lake Nyasa fish—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting BCD.152/4/01/T.

INSTRUMENT MAKERS or TOOLMAKERS, for varied and interesting work in a small workshop engaged on scientific research—The Administrator, Medical Research Council, Radiobiological Research Unit, Harwell, Didcot, Berkshire.

LECTURER (with a good honours degree in physics and preferably a Ph.D. degree, experimental research experience in some aspects of plasma physics and an interest in high-current research work) IN THE DEPARTMENT OF NATURAL PHILOSOPHY—The Secretary-Treasurer, Royal College of Science and Technology, George Street, Glasgow, C.1.

PHYSICISTS or ENGINEERS IN THE NUCLEAR PHYSICS DEPARTMENT, to assist in the bubble chamber development programme—Prof. D. H. Wilkinson, Clarendon Laboratory, The University, Oxford.

RESEARCH ASSISTANT (with experience of numerical analysis and of high-speed computers) IN THE MATHEMATICS DEPARTMENT, to be responsible for the day-to-day administration of a National-Elliott 803 digital computer, assist potential users, both from within the College and from outside, and to formulate and programme their problems—Chief Education Officer, Brunel College of Technology, Woodlands Avenue, Acton, London, W.3.

RESEARCH ASSISTANTS IN (*a*) METALLURGY and (*b*) CHEMISTRY—The Registrar, Bradford Institute of Technology, Bradford 7.

RESEARCH ASSOCIATE and a RESEARCH ASSISTANT, to join in an investigation of the synthesis and degradation of sulphate esters in mammalian tissues—Prof. Brian Spencer, Trinity College, Dublin, Republic of Ireland.

RESEARCH FELLOW or ASSISTANT (preferably with experience in programming for a digital computer) IN THE DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS, for work in the field of hypersonic gas dynamics—The Secretary and Registrar, The University, Southampton.

SCIENTISTS, SCIENTIFIC OFFICERS or SENIOR SCIENTIFIC OFFICERS (with a first- or second-class honours degree or equivalent, particularly in chemistry, physics, mathematics or engineering), for work in research and development establishments in the South of England—The Chief Scientist, The War Office, BN., London, S.W.1.

SENIOR AGRICULTURAL OFFICER (under 55, with a degree or diploma in horticulture or agriculture, a good knowledge of the production of tropical fruit and food crops and temperate vegetables, and preferably a knowledge of applied entomology and agricultural extension methods) in the Bahamas to encourage local production of subsistence crops and tropical fruits and advise on control measures of plant pests—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting RC.213/8/01/T.

SENIOR LECTURER IN SOLID MECHANICS IN THE DEPARTMENT OF AIRCRAFT DESIGN—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

SENIOR RESEARCH ASSISTANT (with a good honours degree in physics or chemical or mechanical engineering) IN THE DEPARTMENT OF FUEL TECHNOLOGY AND CHEMICAL ENGINEERING—The Registrar, The University, Sheffield.

SENIOR TECHNICAL ASSISTANT (preferably with experience in the use of transistors, familiarity with nuclear instrumentation, and some knowledge of workshop practice) IN THE PHYSICS DEPARTMENT, to help in the design and construction of electronic and other instruments to be used in rockets and artificial Earth satellites—The Secretary and Registrar, The University, Southampton.

SENIOR TECHNICIAN IN THE CHEMISTRY DEPARTMENT, to organize and develop the department stores, and to prepare for their expansion in a new building to be occupied in 1965—The Registrar (CSTS), Queen Mary College (University of London), Mile End Road, London, E.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Lister Institute of Preventive Medicine. Report of the Governing Body, 1961. Pp. 29. (London: The Lister Institute of Preventive Medicine, 1961.) [67

The Natural Rubber Producers' Research Association. Twenty-third Annual Report. Pp. 49. (London: The Natural Rubber Producers' Research Association, 1961.) [67

Review of Coal Tar Technology, Vol. 12, Part 2: July–December 1960. Pp. 75–158. (Gomersal, Leeds: Coal Tar Research Association, 1961.) 20*s*. per volume. [67

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